

ARCHIVES

OF

INTERNAL MEDICINE

EDITORIAL BOARD

JOSEPH L MILLER, Chicago
RICHARD C CABOT, Boston LOUIS V HAMMAN, Baltimore
GEORGE DOCK, St Louis WARFIELD T LONGCOPE, New York City
W S THAYER, Baltimore

VOLUME 28

1921

CHICAGO
AMERICAN MEDICAL ASSOCIATION
PUBLISHERS

CONTENTS OF VOLUME 28

JULY, 1921 NUMBER 1

	PAGE
RENAL IRRITATION IN MAN FROM HIGH PROTEIN DIET THEODORE L SQUIER, M D, AND L H NEWBURGH M D, ANN ARBOR, MICH	1
II THE EFFECT OF CYANIDS AND OF ORGANIC OXIDIZING AGENTS ON THE LIVER INJURY CAUSED BY CHLOROFORM N C DAVIS, M D, SAN FRANCISCO	20
ON THE ETIOLOGY OF AN OUTBREAK OF INFECTIOUS DIARRHEA HARRY WEISS, BOSTON	37
THE INTRAVENOUS USE OF FOREIGN PROTEIN IN THE TREATMENT OF CHRONIC CASES OF ARTHRITIS, WITH SPECIAL REFERENCE TO THE USE OF SECONDARY PROTEOSE R GARFIELD SNYDER, M D, AND MAXIMILIAN A RAMIREZ, M D, NEW YORK	50
RENAL GLYCOSURIA PHIL L MARSH, M D, ANN ARBOR, MICH	54
THE RIGHT VENTRICLE IN PULMONARY TUBERCULOSIS ERNST P BOAS, M D, AND HUBERT MANN, M D, NEW YORK	62
FREQUENT CAUSES AND THE TREATMENT OF SEASONAL HAY-FEVER I CHANDLER WALKER, M D, BOSTON	71
SOME URINARY CHANGES IN NORMAL INDIVIDUALS ON THE PELLAGRA PRODUCING EXPERIMENTAL DIET M X SULLIVAN, SPARTANBURG, S C	119
BOOK REVIEW	124

AUGUST, 1921 NUMBER 2

OBJECTS AND METHOD OF DIET ADJUSTMENT IN DIABETES R T WOODYATT, M D, CHICAGO	125
ACUTE YELLOW ATROPHY OF THE LIVER MAX KAHN, M D, PH D, AND JOSEPH BARSKY, M D, NEW YORK	142
FOOD ALLERGY AS A CAUSE OF ABDOMINAL PAIN WILLIAM W DUKE, M D, KANSAS CITY, MO	151
PHENOLS IN THE URINE IN PELLAGRA M X SULLIVAN AND PAUL R DAWSON, SPARTANBURG, S C	166
THE BASAL METABOLISM AND THE SPECIFIC DYNAMIC ACTION OF PROTEIN IN LIVER DISEASE JOSEPH C AUB, M D, AND JAMES H MEANS, M D, BOSTON	173
THE DIAGNOSIS OF EVENTRATION OF THE DIAPHRAGM WITH A REPORT OF A CASE OF APLASIA OF THE RIGHT LUNG AND RIGHT HALF OF THE DIAPHRAGM, ASSOCIATED WITH CONGENITAL DEXTROCARDIA HORACE MARSHALL KORN, M D, CLEVELAND	192
THE BIGEMINAL PULSE IN ATRIOVENTRICULAR RHYTHM PAUL D WHITE, M D, BOSTON	213
THE SYNTHESIS AND ELIMINATION OF HIPPURIC ACID IN NEPHRITIS A NEW RENAL FUNCTION TEST PRELIMINARY PAPER F B KINGSBURY, PH D, AND W W SWANSON, B S, MINNEAPOLIS	220
BOOK REVIEW	237

CONTENTS OF VOLUME 28

SEPTEMBER, 1921 NUMBER 3

	PAGE
EPITHELIOMATA OF THYMIC ORIGIN DOUGLAS SYMMERS, M D, AND B M VANCE, M D, NEW YORK	239
BETELNUT CHEWING AND ITS EFFECTS, INCLUDING CANCER OF THE MOUTH ALLER G ELLIS, M D, BANGKOK, SIAM	252
CAROTINEMIA REPORT OF A CASE IN AN ADULT GEORGE DOUGLAS HEAD, M D, AND REUBEN A JOHNSON, M D, MINNEAPOLIS	268
FOCAL INFECTION AND ELECTIVE LOCALIZATION IN THE ETIOLOGY OF MYOSITIS EDWARD C ROSENOW, M D, AND WINIFRED ASHBY, PH D, ROCHESTER, MINN	274
ON THE CAUSES OF THE VARIATIONS IN THE SEDIMENTATION OF THE CORPUSCLES AND THE FORMATION OF THE CRUSTA PHLOGISTICA ("SIZE," "BUFFY COAT") ON THE BLOOD A PRELIMINARY COMMUNICATION H C GRAM, COPENHAGEN, DENMARK	312
OBSERVATIONS ON THE RELATION OF UREA TO UREMIA LOUIS LEITER, CHICAGO	331
METABOLIC STUDIES ON A CASE OF DIABETES INSIPIDUS I M RABINOWITCH, M D, MONTREAL, CANADA	355

OCTOBER, 1921 NUMBER 4

STUDIES IN OXYGEN THERAPY WITH DETERMINATIONS OF THE BLOOD GASES I IN CARDIAC INSUFFICIENCY AND RELATED CONDITIONS ALVAN L BARACH, M D, AND MARGARET N WOODWELL, A B, BOSTON	367
STUDIES IN OXYGEN THERAPY II IN PNEUMONIA AND ITS COMPLICATIONS ALVAN L BARACH, M D, AND MARGARET N WOODWELL, A B, BOSTON	394
STUDIES IN OXYGEN THERAPY III IN AN EXTREME TYPE OF SHALLOW BREATHING OCCURRING IN LETHARGIC ENCEPHALITIS ALVAN L BARACH, M D, AND MARGARET N WOODWELL, A B, BOSTON	421
THE TOTAL NONPROTEIN NITROGEN CONSTITUENTS OF THE BLOOD IN CHRONIC NEPHRITIS WITH HYPERTENSION J LISLE WILLIAMS, CHICAGO	426
A CONTRIBUTION TO THE STUDY OF TUMORS FROM THE PRIMITIVE NOTOCHORD N D C LEWIS, M D, WASHINGTON, D C	434
FURTHER OBSERVATIONS ON EXPERIMENTAL LESIONS OF THE BRANCHES OF THE AURICULOVENTRICULAR BUNDLE OF THE DOG FRED M SMITH, M D, CHICAGO	453
PRIMARY HEMANGIOLYMPHOMA OF THE HEMAL NODES, AN UNUSUAL VARIETY OF MALIGNANT TUMOR DOUGLAS SYMMERS, M D, NEW YORK	467
THE CHEMISTRY AND CLINICAL SIGNIFICANCE OF UROBILIN LOUIS BAUMAN, M D, NEW YORK	475
A STUDY OF THE S-T INTERVAL IN ONE THOUSAND AND TWENTY-EIGHT ELECTROCARDIOGRAMS L ARTHUR BUCHANAN, ROCHESTER, MINN	484
PERFORATION OF THE INTERVENTRICULAR SEPTUM OF THE HEART WITH REPORT OF A CASE JOHN B YOUNG, M D, BOSTON	495

CONTENTS OF VOLUME 28

NOVEMBER, 1921 NUMBER 5

	PAGE
CLINICAL STUDIES OF THE RESPIRATION VII THE EFFECT OF GENERAL WEAKNESS AND FATIGUE ON THE VITAL CAPACITY OF THE LUNGS FRANCIS W PEABODY, M D, AND CYRUS C STURGIS, M D, BOSTON	501
STUDIES ON ERYTHROCYTES, WITH SPECIAL REFERENCE TO RETICULUM, POLYCHROMATOPHILIA AND MITOCHONDRIA J ALBERT KEY, M D, BOSTON	511
UNCINARIAL NEPHRITIS F ROJAS, M D, AND J TANCA MORENGO, M D, GUAYAQUIL, ECUADOR, S A	550
COMPARATIVE STUDIES IN THE CHEMISTRY OF BLOOD AND CEREBROSPINAL FLUID GRETE EGERER-SEHAM, PH D, AND C E NIXON, M D, MINNEAPOLIS	561
AN ANALYSIS OF NINETY CASES OF FUNCTIONAL DISEASE IN SOLDIERS JOHN M SWAN, M D, ROCHESTER, N Y	586
VARIATION OF THE PHENOLSULPHONEPHTHALEIN EXCRETION WITH THE URINE VOLUME IN CHRONIC INTERSTITIAL NEPHRITIS R R SNOWDEN, M D, PITTSBURGH	603
RELATION OF THE GASTRIC CONTENT TO THE SECRETORY AND MOTOR FUNCTIONS OF THE STOMACH HOMER WHEELON, M D, ST LOUIS	613
A CASE OF ALKAPTONURIA WITH A STUDY OF ITS METABOLISM R B GIBSON, PH D, AND C P HOWARD, M D, IOWA CITY	632
THE INFLUENCE OF INORGANIC IRON ON THE REGENERATION OF BLOOD AFTER HEMORRHAGIC ANEMIA JOHN H MUSSER, JR, M D, PHILADELPHIA	638
A NEW METHOD OF INTERPRETATION OF THE RENAL FUNCTION TEST MEAL HERMAN SHARLIT, M D, AND WILLIAM G LYLE, M D, NEW YORK	649
TERMINAL CARDIAC ARRHYTHMIAS REPORT OF THREE CASES FRANCIS R DIEUAIDE, M D, AND EDWARD C DAVIDSON, M D, BALTIMORE	663
EXPERIMENTAL INQUIRY INTO THE CEREBRAL AND NEUROMUSCULAR MANIFESTATIONS OF DIGITALIS D I MACHT, M D, AND WM BLOOM, BALTIMORE	678

DECEMBER, 1921 NUMBER 6

THE BLOOD IN TETRACHLORETHANE POISONING GEORGE R MINOT, M D, AND LAWRENCE W SMITH, M D, BOSTON	687
THE EFFECT OF THE EXTRACT OF THE POSTERIOR LOBE OF THE PITUITARY ON BASAL METABOLISM IN NORMAL INDIVIDUALS AND IN THOSE WITH ENDOCRINE DISTURBANCES C A MCKINLAY, M D, MINNEAPOLIS	703
CAPILLARY POISONS AND ACIDOSIS GEORGE B WALLACE, M D, AND EMIL J PELLINI, M D, NEW YORK	711
LOCAL DESENSITIZATION IN HYPERSENSITIVE INDIVIDUALS AND ITS BEARING ON THE PREVENTION OF HAY-FEVER GEORGE M MACKENZIE, M D, AND LOUIS B BALDWIN, M D, NEW YORK	722
PRIMARY CARCINOMA OF THE LUNGS T S MOISE, M D, NEW HAVEN, CONN	733

CONTENTS OF VOLUME 28

	PAGE
EXPERIMENTAL DIABETES INSIPIDUS PERCIVAL BAILEY, BOSTON, AND FREDERIC BREMER, BRUSSELS	773
OBSERVATIONS ON GLYCEMIA, GLYCURESIS AND WATER EXCRETION IN OBESITY CAROL BEELER, B S, AND REGINALD FITZ, M D, ROCHESTER, MINN	804
A STUDY OF SIGNIFICANT CHEMICAL CHANGES IN THE BLOOD COINCIDENT WITH MALIGNANT TUMORS JOHN A KILLIAN, PH D, AND LUDWIG KAST, M D, NEW YORK	813
A STUDY OF THE UREA CONCENTRATION TEST FOR KIDNEY FUNCTION I M RABINOWITCH, M D, MONTREAL	827
THE ACTION OF THE NITRITES ON THE CORONARY CIRCULATION FRED M SMITH, M D, CHICAGO	836
A SIMPLE AND ACCURATE METABOLISM SPIROMETER SPIROMETER MEASUREMENTS OF OXYGEN CONSUMPTION BY THE REBREATHING METHOD CHARLES CLAUDE GUTHRIE, M D, PH D, PITTSBURGH	841
THE EFFECT OF THE INGESTION OF FOODSTUFFS ON THE RESPIRATORY EXCHANGE IN PULMONARY TUBERCULOSIS WILLIAM S McCANN, M D,, WITH THE TECHNICAL ASSISTANCE OF G F SODERSTROM, NEW YORK	847
INDEX TO VOLUME 28	859

RENAL IRRITATION IN MAN FROM HIGH PROTEIN DIET *

THEODORE L SQUIER, M D, AND L H NEWBURGH, M D
ANN ARBOR, MICH

In 1918 it was shown by one of us ¹ that a high protein diet over long periods produced in rabbits chronic, diffuse, bilateral kidney lesions. It seemed not improbable, then, to expect some evidence of renal irritation if high protein diets were forced for short periods in those cases where some kidney damage already might exist.

In recent years a number of investigators have become more and more convinced that cases of so called essential hypertension were in fact nephritic. Harpuder ² made a careful clinical and anatomic study of cases of essential hypertension and concluded that in every case a persistent hypertension was indicative of chronic nephritis of "arteriosclerotic type." Clinically, out of 504 cases with a systolic blood pressure persistently over 160 mm mercury, 490, or 97.2 per cent showed definite disturbances of kidney function. But this view is not as yet universally accepted. Moschowitz, ³ for example, maintains that hypertension is not an outcome of nephritis but rather the response to continuous circulatory vasoconstricting influences and the kidney lesions result from the hypertension.

Inasmuch as the usual functional kidney tests fail in a certain percentage of cases of hypertension to give any evidence of kidney damage, we attempted to obtain such evidence by throwing an extra load on that organ. It seemed not improbable to expect some evidence of renal irritation if protein diets were forced for short periods in those cases in which some kidney damage already might exist.

PART 1

The patients used in the first part of this study were ordinary ward patients whose chief complaints centered about symptoms resulting from hypertension. For obvious reasons, the number of cases studied

* From the Department of Internal Medicine, Medical School, University of Michigan.

1 Newburgh, L. H. The Production of Bright's Disease by Feeding High Protein Diets, *Arch Int Med* **24** 359 (Oct) 1919.

2 Harpuder, K. Arteriosclerose, Schrumpfnieren u. Blutdruck, *Deutsch Arch f klin Med* **129** 44, 1918.

3 Moschowitz, E. Hypertension. Its Significance, Relation to Arteriosclerosis and Nephritis and Etiology, *Am J M Sc* **158** 668, 1919.

was kept as small as was deemed consistent with conclusive results. Phenolsulphonephthalein elimination varied in the cases studied between 20 and 45 per cent in two hours, and the blood urea nitrogen, determined by the Van Slyke method, varied between 9.8 and 13.0 mg. Preceding the high meat diet each patient was put on a low protein salt free diet for a period of from 5 to 10 days, during which time careful daily urinalyses were made. In Case 1, a trace of albumin was persistently present in the urine, but in the other cases albumin was absent. Occasional hyaline casts were found in all. In no case were red blood corpuscles found at any time preceding the high meat diet, and it should be emphasized at this point that in every case a careful daily search, with a 4 mm objective, was made for such cells. A ward record was kept of the amount of protein actually eaten. During the low protein period this averaged 33 gm in twenty-four hours, while on the forced protein diet from 100 to 175 gm protein was ingested each day. A summary of the cases so studied follows.

REPORT OF CLINICAL OBSERVATIONS

CASE 1—Female, aged 48, entered the hospital complaining of dizziness, periods of syncope, hot flashes and black spots before the eyes. She was married at 18, and had no pregnancies. The menopause occurred at 47. There was no history of scarlet fever or sore throat. She has had frontal headaches for the last twenty-five years. Nocturia has been present three times each night since childhood. Several months ago, systolic pressure was 260 mm.

Physical Examination—On examination, the teeth were found to be carious and the tonsils atrophic. The left border of the heart was 10.5 cm to the left of the midsternal line. No murmurs were heard at the apex. At the aortic area there was a high-pitched shrill crescendo systolic murmur ending in a bell-like second sound. The orthodiagram revealed a tortuous arch with some pushing downward of the heart but the heart area was normal. Very slight edema of both ankles was present. Fundus examination showed neuroretinitis with hemorrhages in the retina and degenerative changes about the macula. Her urine at entrance showed a faint trace of albumin, occasional granular casts, many white blood cells and no red blood cells. Blood pressure was 245/190. Phenolsulphonephthalein elimination was 10 per cent the first hour and 10 per cent the second hour. Blood urea nitrogen was 9.8 mg.

May 27, she was given a Mosenthal test meal. The specific gravity of the urine varied from 1.012 to 1.016, showing definite fixation.

Clinical Observation—On admission she was placed on a salt free diet giving 34 gm protein. After nine days of this diet, with rest in bed, her diastolic pressure had fallen from 190 to 140 mm but the systolic pressure remained practically unchanged.

Starting May 29 she was given a high protein, salt poor diet, containing from 140 to 180 gm protein daily. The urine at the beginning contained 0.5 gm albumin per liter, and many white blood cells and hyaline casts, but no red blood cells. For a long period the urine showed no marked change but the blood urea nitrogen gradually increased, being 12.1 mg after five days of high protein, 21 mg after ten days (June 7) and 25 mg after nineteen days (June 16). June 18, twenty-one days after high protein was started, there was a decided increase in albumin to 2 gm per liter, and three days

later (June 21) a few red blood cells⁴ were found for the first time in her urine. Simultaneously with the urinary changes, definite edema appeared with distinct pitting of the ankles and her weight increased from 123 pounds, June 17, to 129 pounds June 24.

During the period of high protein diet, three fundus examinations were made in the department of ophthalmology. June 1 and June 9 no changes were found in the condition of the fundus as originally reported. June 21 following the

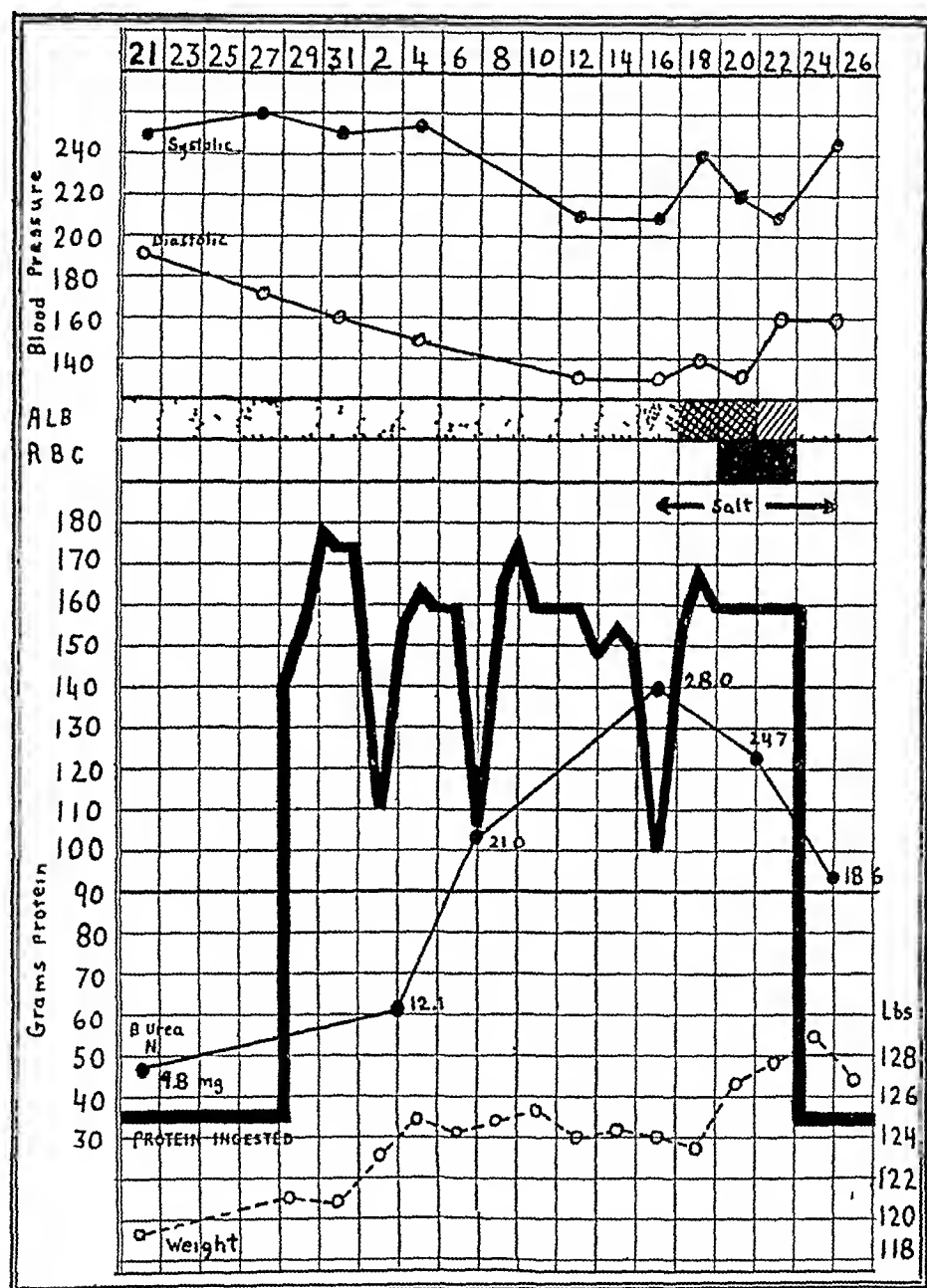


Chart 1—Case 1

appearance of red blood corpuscles in the urine, the fundus examination showed macular changes more advanced than at the previous examinations. June 24, high protein was discontinued, and June 26, only a trace of albumin was present in the urine. No red blood corpuscles were found after June 24. Fol-

⁴ In every case the presence of red blood corpuscles was confirmed by at least two other observers.

lowing discharge, urine specimens were examined July 7 and 30 and August 28, and in each instance neither albumin nor red blood corpuscles were found

Comment—In this case twenty-one days of high protein diet resulted in a definite increase in the amount of albumin present in the urine, in the appearance of red blood corpuscles which had previously been absent, in a definite advance in the macular changes of the fundus as shown by ophthalmoscopic examination, and in the appearance of definite edema evidenced by pitting of the ankles and increase in weight. The changes in diet had no effect on the blood pressure

CASE 2—Male, aged 58, married, lumber scaler. Entered May 22 complaining of shortness of breath and failing vision. The family history and marital history were unimportant

Previous Illness—He had measles in childhood but no scarlet fever or tonsillitis. Ever since early youth he had "rheumatism" in his left knee. He has had frequent attacks of frontal headache which have been especially severe in the last two years. For some time he has had discharge from his left ear and the hearing has been completely lost on this side. He is subject to chronic head colds, and during the last two months he has had occasional attacks of epistaxis. For a number of years there has been nocturia, two to four times. No history of pyuria or hematuria was obtained. His ankles have never been swollen. Recently he has had occasional attacks of dizziness. About two years ago his vision became suddenly blurred and he attributed this to some new glasses which he had just gotten, but several changes of glasses failed to improve it. At about the same time he noticed that he was becoming short of breath on slight exertion and there were occasional attacks of palpitation. This condition has very slowly grown worse up to the present time.

On further questioning, an interesting and important dietary history was obtained. For the past thirty-five years he has worked in a lumber camp, and during the entire time has eaten large quantities of meat. He states that he would average about one-half pound of meat three times daily, and gives as an example of his usual diet: Breakfast, pancakes, fried potatoes, half pound of steak. Noon, roast beef, about one-half pound, potatoes and gravy, corn and tomatoes or some other vegetable. Evening, potatoes and all he could eat of some kind of meat generally from one-half to three-quarters of a pound.

Physical Examination—On examination, the pupils were normal in contour and reaction. There was marked impairment of hearing in the left ear. Many of the teeth were absent and a number of those remaining were carious. Pyorrhea was marked. The lungs were normal. The left border of the heart was 11.5 cm. to the left of the midsternal line, and the apex beat was best heard and felt in the fifth intercostal space. The first mitral sound was reduplicated but no murmurs were present at the apex or base. A₂ was markedly accentuated. The liver edge was palpable three finger breadths below the rib margin in the nipple line. The spleen was not felt. Tendon reflexes were all normal. The department of otolaryngology reported old left sided otitis media of no clinical importance.

Ophthalmologic examination showed neuroretinitis with some edema of the nerve head and some old retinal hemorrhages. The blood Wassermann was negative. Red blood corpuscles 5,200,000, hemoglobin, 100 per cent (Talqvist), white blood corpuscles 8,650. The blood pressure on admission was 200/120. Phenolsulphonephthalein excretion was 15 per cent in the first hour, 20 per cent in the second hour. Blood urea nitrogen was 13 mg. per 100 c.c. From the day of admission May 22 until May 28 he was kept on a salt poor diet.

containing about 35 gm protein, and during this period careful repeated urine examinations were made. A few white blood cells and an occasional cast were found but at no time was albumin present nor were red blood corpuscles found.

May 27 he was given a Mosenthal meal and variation of urine, specific gravity from 1008 to 1020 followed. The quantity of night urine was, however, definitely increased. After seven days of low protein diet his blood urea nitrogen had reached the low level of 79 mg per 100 cc.

Clinical Observation—Beginning May 29 his protein intake was increased to the maximum. This was 220 gm protein the first day and 182 gm the following day. The first day after high meat diet was started (May 30) albu-

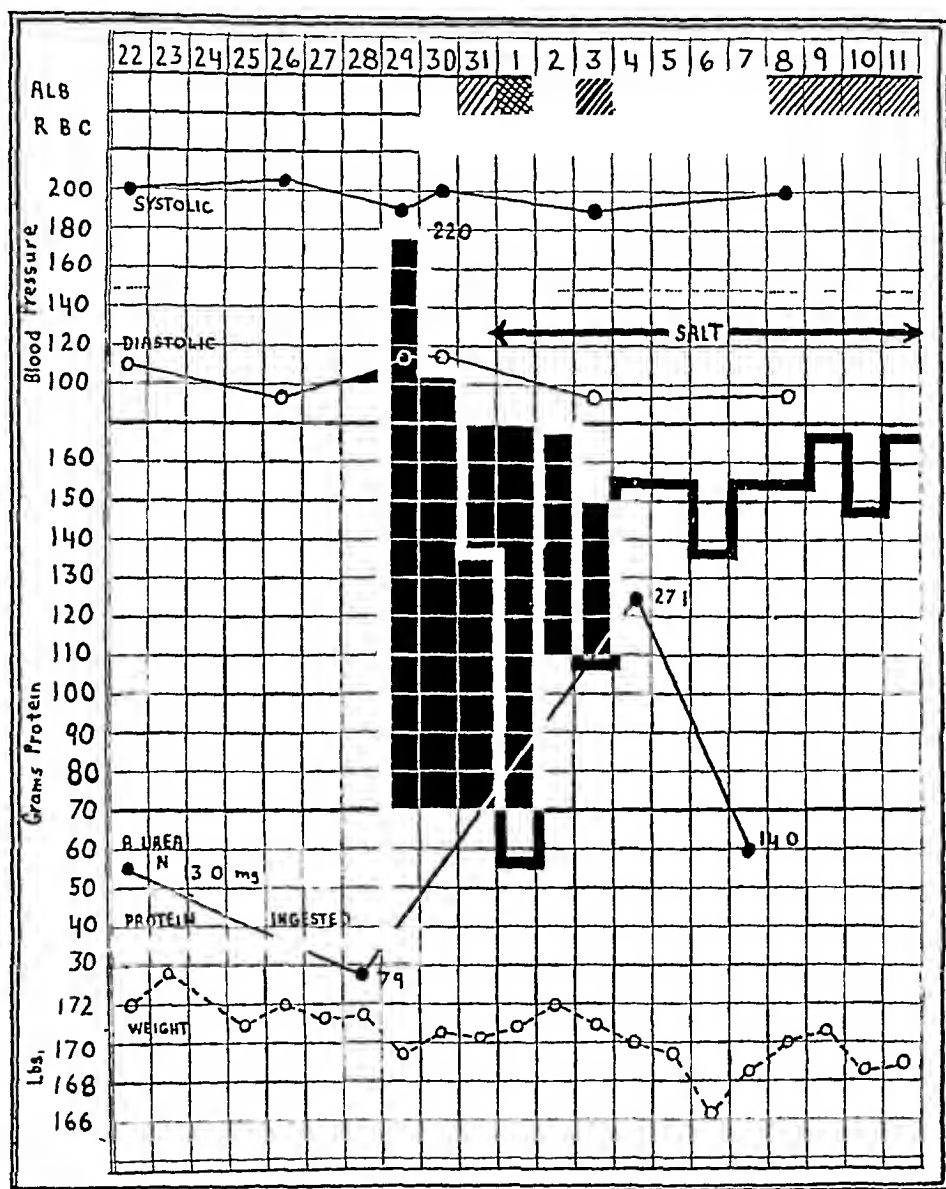


Chart 2—Case 2

min was present and a few red blood corpuscles were found. The daily quantity of albumin as determined by the Esbach tube gradually increased from 0.2 gm per liter to 0.8 gm per liter on the third day (June 1). On this day salt was added to his diet, and the following day the urine was found to contain 17 gm albumin per liter. High protein diet without salt limitation was continued for ten days (until June 11). During this period there was no increase in weight and no evidence of edema. The blood urea nitrogen

reached a maximum of 271 mg June 4, after which it fell, until June 7 it was 14 mg. The phenolsulphonephthalein excretion showed a striking increase June 11, the phenolsulphonephthalein elimination for the first hour was 50 per cent as contrasted with 15 per cent at the time of admission, and for the second hour it was 25 per cent as contrasted with 20 per cent. Assuming the first determination to be correct, inasmuch as the technic was the same in both instances, the marked increase in phenolsulphonephthalein elimination would seem to indicate renal irritation. Possibly, the fall noted in blood urea nitrogen may be explained on the same basis. During the period of high protein feeding, daily urinalyses invariably showed from two to six red blood corpuscles to the high power field. June 2, the fourth day after red blood corpuscles were found in the urine, the fundi were reexamined in the department of ophthalmology. They reported as follows:

S shows a well defined increase of edema in the cup of the right eye, and an increase of hemorrhages both deep and superficial in the left eye. These changes may be construed to be resultant from the same factor or factors causing the original changes noted before.

Comment—In this case high protein feeding resulted in almost immediate appearance of red blood corpuscles in the urine when they had previously been absent, in definite albuminuria which previously had not been present, in a rise in the blood urea nitrogen, later followed by a fall, in a marked increase in phenolsulphonephthalein elimination, which in conjunction with the secondary fall in blood urea nitrogen is interpreted as possible evidence of renal irritation, and in a distinct increase in retinal hemorrhages.

CASE 3—Scotch drayman, aged 61, married, entered the hospital, August 11, complaining of occipital headaches and dizzy spells. His mother died at 67 of dropsy, and one brother died of Bright's disease. Otherwise the family history is unimportant.

Previous Illness—He had scarlet fever and diphtheria in early youth but no other serious illness. For many years he has been troubled with headaches and for the past year his vision has been failing. Venereal disease was denied. There have been occasional attacks of sore throat and for the last two years he has had slight asthmatic attacks.

Present Illness—He dates the onset of the present illness to six months ago at which time he had severe occipital headache accompanied by swelling just above and in front of each ear. Attacks of headache were easily brought on by excitement or exposure to the sun. About two months ago, the pain began to grow worse, and it has steadily progressed since that time, being worse in the morning on arising. There have recently been occasional dizzy spells when he feels as though the blood had "rushed to his head." He has noticed that, at times, large amounts of urine are passed, while at other times the amount is but slight. There has been only occasional nocturia.

Physical Examination—On examination the teeth were found to be in fair condition, except for marked pyorrhea. The lungs were negative. The heart was slightly enlarged to the left, and at the apex there was a soft blowing systolic murmur faintly transmitted to the axilla. The aortic second sound was accentuated. The radials and brachials were slightly firmer than normal and there was a suggestion of beading. Abdominal examination was negative. No focus of infection could be demonstrated in the nose, throat or ears by the otolaryngology department, and a roentgenogram of the sinuses was normal.

An examination in the department of ophthalmology showed angiopathic retinitis, hyperemia of the nerve head and angiosclerotic changes of both eyes with some choroidal changes in the left eye

The blood Wassermann was negative, and the hemoglobin, red and white cell counts were all normal. At the time of admission a reducing substance was found in the urine which was otherwise normal, and he was consequently placed on the routine green diet. At no subsequent examination was any reducing substance found. The blood pressure on admission was 195/125. The phenolsulphonephthalein excretion was 10 per cent in the first hour and 14 per cent in the second hour. The blood urea nitrogen, determined after eight days of low protein diet, was 112 mg per 100 cc.

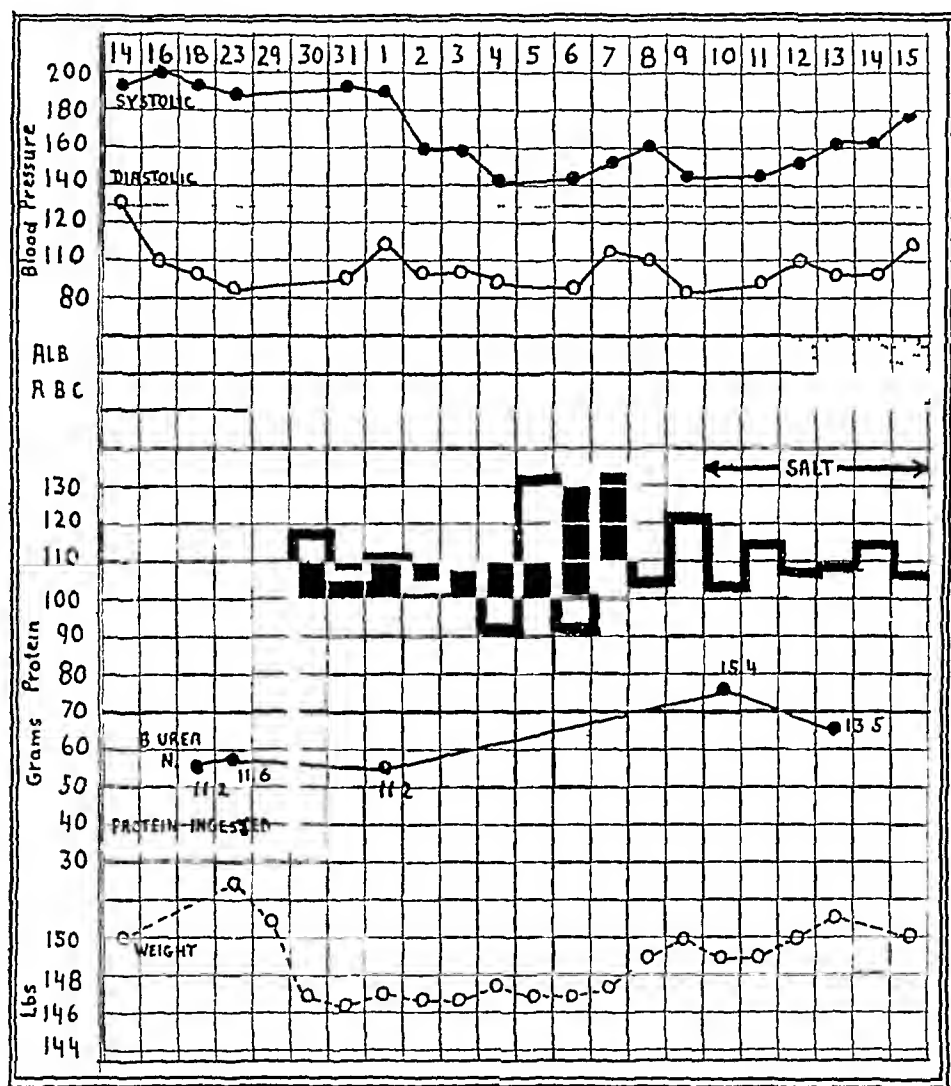


Chart 3—Case 3

Clinical Observation—August 30, after eighteen days of low protein diet, during which time an occasional hyalin cast was the only urinary abnormality found on repeated careful examinations, he was given a high protein diet. It was impossible to induce him to eat as much protein as in either of the preceding cases. After two days of high protein, the urine which had previously been free from albumin contained a distinct trace, which persisted for five days and then disappeared. September 10, after twelve days of forced protein feeding, the blood urea nitrogen was 154 mg per 100 cc. During the entire time no red blood corpuscles were observed but occasional hyaline casts and a few white blood cells were found each day.

September 11, after thirteen days of high protein, salt was allowed in addition and on the following day, September 12, a trace of albumin was again found in the urine. September 13, the fifteenth day of high protein diet and after two days of unrestricted salt, from one to two red blood corpuscles to the high power field were found in the urine and on the following day the number of red cells was distinctly increased. September 15, two days after red cells were found, an ophthalmoscopic examination was again made in the department of ophthalmology and the following report returned:

There is considerably more edema of the disk in the right eye, the rings barely showing, the disk is hyperemic, the veins are engorged and the retina has more loss of transparency.

In the left eye the disk is likewise more edematous than at the previous examination and is hyperemic. The veins are engorged. The retina has marked loss of transparency near the disk and in the macular region. Both eyes present a definite increase in edema. No hemorrhages were seen in either eye.

He was discharged September 15 before a phenolsulphonephthalein determination could be repeated. It is interesting, however, to note that the blood urea nitrogen September 13 was 135 mg—distinctly lower than on September 10 before red cells had appeared in the urine.

October 10, a urine specimen submitted for examination showed a trace of albumin but no red blood corpuscles were present.

Comment—Here, again, prolonged high protein feeding was followed by definite evidence of renal disturbance. Shortly after the initial irritation of high protein feeding, a trace of albumin was found in the urine which had previously been albumin free. After five days of the same diet the urine again became albumin free, but shortly after salt was added to the diet it reappeared. An hypothesis at least worthy of consideration is that subsequent to throwing an added load on the kidneys suddenly, there was albuminuria which disappeared as soon as the kidneys became adjusted to the added task. Again, the further addition of salt to the diet temporarily overtaxed the kidneys with resulting reappearance of albumin.

After continued high protein diet, during the last three days of which salt was allowed, red blood corpuscles appeared in the urine which had previously been free from such cells at each daily examination.

The fundus examinations revealed a moderate but distinct increase in the engorgement and edema of the retina subsequent to the high protein feeding.

No significant change in blood pressure was noted during the period of forced protein diet.

CASE 4—Woman, aged 56. American, housewife, married, entered the hospital August 26, complaining of pain in the back, shortness of breath, palpitation and nocturia. One sister died of Bright's disease, but otherwise the family history is unimportant.

Previous History—She had the usual children's diseases with diphtheria at 13 after which she had repeated attacks of sore throat. At 19, following the birth of her first child, she was in bed four or five weeks with scarlet fever complicated by left otitis media. Fifteen years ago she was sick five weeks

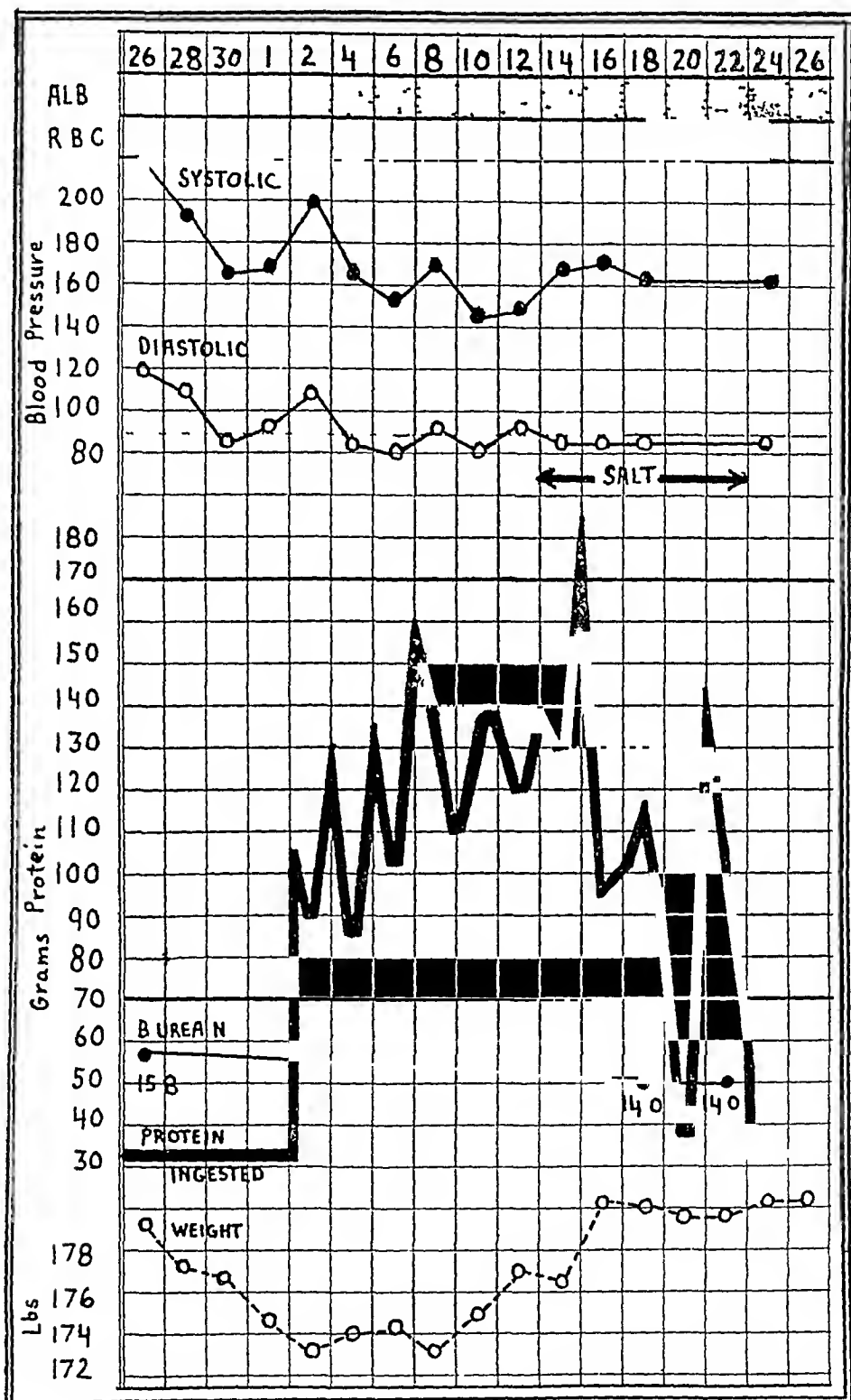


Chart 4—Case 4

with what she describes as "congestion of the kidney." At this time there was difficulty in urination and the urine was dark, scant and strong smelling. Her face and ankles were swollen and her mouth very sore. The attack was not associated with any other illness. Four years ago, she had "sciatica." December, 1918 she had influenza and was in bed two weeks after which her throat remained sore for five weeks. December 1919, she was in bed for three weeks complaining of shortness of breath, tired feeling and pain in the back. During this period she says there were three days during which she passed no urine.

Present Illness—Her present illness really dates from her trouble fifteen years ago, but since her attack of influenza, although her symptoms have not been markedly worse she has felt more concerned about them. Last March she began to have occasional attacks of sharp pain in the region of the sternum which radiated to the left shoulder. Following an attack she would perspire and feel weak. For several years there has been nocturia twice each night.

Physical Examination—On examination, the pupils were normal, the tonsils slightly enlarged and the postcervical and submental glands were palpable. There were a few crackles at the base of the right lung, but otherwise the chest was negative. Because of obesity, the heart apex could not be determined very well, but there was no obvious enlargement. The heart sounds were faint, and there was a soft blowing systolic murmur at the apex not well transmitted to the axilla. The aortic second sound was accentuated. The abdomen was negative and except for many varicose veins in the legs, the extremities were negative. There was no edema.

The department of otolaryngology reported small buried tonsils with slight hypertrophic rhinitis. Although a roentgenogram of the head showed some clouding of the accessory sinuses, it was felt that there was no evidence of present active infection. A roentgenogram of the chest showed nothing abnormal, and the heart was reported to be normal in size and shape. The department of ophthalmology reported bilateral angiosclerosis, neuroretinitis, venous engorgement and marked edema. There was also central punctate retinitis.

Her blood Wassermann was negative, and the blood count was normal. The blood pressure on entrance was 220/120. Blood urea nitrogen on admission was 15.8 mg per 100 c.c. Phenolsulphonephthalein elimination for the first hour was 30 per cent, for the second hour, 15 per cent. A few white blood cells and occasionally hyaline casts were found in the urine. There were no red blood cells and no albumin was present.

Clinical Observation—For seven days following admission she was kept in bed on low-protein, salt-free diet. During this time there was a tendency for her blood pressure to fall. September 2, forced high protein feeding was begun. The initiation of high protein feeding made no change in the general downward trend of her blood pressure. September 3 a trace of albumin was found in the urine and this persisted in an amount practically unchanged during her stay in the hospital. September 14, after twelve days of high protein diet, salt was allowed in addition to the high meat, and September 19, after seventeen days of high protein feeding and five days after the addition of salt, rare red blood corpuscles were found in the urine. Red cells were found in the urine in small numbers for four succeeding days until the high protein diet was discontinued. September 25, after two days of low protein diet, there was still a trace of albumin but no red cells were found. During the high protein feeding the level of blood urea nitrogen remained practically constant, being 14 mg per 100 c.c. September 17 and again September 22. On this last day, the phenolsulphonephthalein elimination was 22 per cent for the first hour and 24 per cent for the second hour. Ophthalmoscopic examination made September 23, three days after red cells were found, showed practically the same appearance reported at the first examination.

Comment—It is seen in this case that an initial hypertension gradually fell regardless of the protein intake. The urine, which had previously been free from albumin, contained a distinct trace on the second day of the high protein diet. Red blood corpuscles appeared in the urine after a long continued period of high protein diet during the

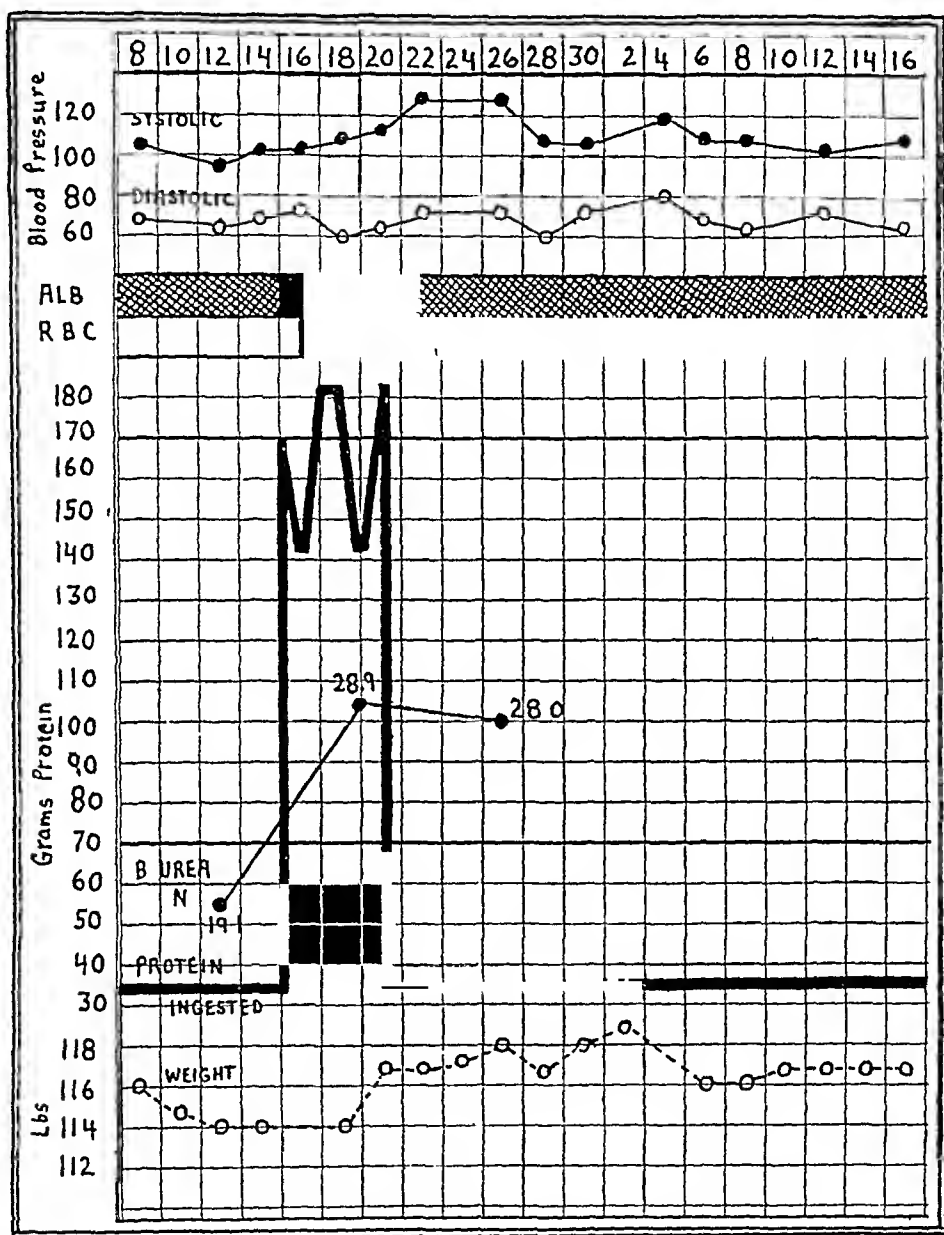


Chart 5—Case 5

last days of which there was no salt restriction. The phenolsulphonephthalein output and blood urea nitrogen remained practically constant during the high protein feeding.

The appearance of red blood corpuscles in the urine of these patients after high protein feeding appeared to us so interesting that we were led to study the effect of such diet in undoubted nephritis.

CASE 5—Male, aged 38, married, entered the hospital June 7, complaining of edema of the face and ankles. The family history was unimportant.

Previous History—There was no history of scarlet fever or tonsillitis and the past history was negative until four years ago when he had psoriasis. About April, 1920, after a series of roentgen-ray treatments for his psoriasis he noticed that he was becoming edematous. His face was first affected, and then his ankles. The onset was gradual and became progressively worse. He was treated for nephritis in a hospital for three weeks and felt somewhat improved at the time of his discharge. He entered this hospital on the advice of his home physician.

Physical Examination—On examination, the pupils were found to be normal in contour and reaction. There was marked pyorrhea. The tonsils were enlarged and somewhat injected, and there was moderate cervical adenitis. Over the chest, abdomen, knees and elbows there was evidence of psoriatic lesions. The lungs and heart were normal. The liver edge could be felt one finger-breadth below the rib margin in the nipple line. There was no demonstrable edema of the ankles and the tendon reflexes were normal.

The day following admission the department of ophthalmology reported neuroretinitis with edema in both eyes. The department of otolaryngology reported hyperseptic tonsils and nasal obstruction, advising tonsillectomy and submucous resection. Dental films showed a moderate generalized pyorrhea but no evidence of a focus. However, six carious teeth were found by the oral surgeon and immediately removed.

The blood Wassermann was negative. Hemoglobin, 90 per cent (Talqvist), red blood corpuscles, 4,000,000, white blood corpuscles, 9,400. Blood pressure was 105/70. The urine showed a definite cloud of albumin on heating but not enough to give a flocculent precipitate and there were a number of white blood corpuscles and occasional hyalin and granular casts but no red blood corpuscles.

Clinical Observation—On admission he was placed on a low-protein, salt-free diet. The phenolsulphonephthalein elimination was 47 per cent for the first hour and 18 per cent for the second hour. June 12, after five days of low protein diet, the blood urea nitrogen was 191 mg per 100 cc.

June 16 forced high protein feeding was started. On the following day (June 17), after having ingested 170 gm protein on the preceding day, from three to five red blood corpuscles were found in the urine per high power field. After three days of high protein (June 19) the blood urea nitrogen had risen to 289 mg. High protein was continued until June 21, and daily urine examinations invariably showed numbers of red blood cells. The amount of albumin which had previously been too small to give a flocculent precipitate, was markedly increased and determinations made by the Esbach method showed 4 gm per liter during the high protein period. June 18, the day following the appearance of red blood cells in the urine and three days after the initiation of high protein diet, the eyes were reexamined in the department of ophthalmology and no change was reported. June 21, after five days of high protein the ophthalmoscopic examination was again repeated. This time an "increase in engorgement and edema" of both eyes was found.

June 26 after he had again been on low-protein salt-poor diet for five days, the albumin in the urine had diminished to 0.5 gm per liter but red blood cells were still found in the urine. Phenolsulphonephthalein elimination determined at this time was 46 per cent for the first hour and 26 per cent for the second hour while the blood urea nitrogen still remained high, being 28 mg per 100 cc.

He remained in the hospital on a low-protein salt-poor diet until July 19, during which time the albumin content of the urine varied between 0.5 and 1.8 gm per liter. Red blood cells were found in the urine at every examination. July 3, tonsillectomy was done. July 29, after he had been on a low-

protein salt-free diet for thirty-eight days, he returned for reexamination and the department of ophthalmology reported "edema of right nerve head slightly less than at previous examination (June 23) No hemorrhages Edema of left nerve head considerably less than at last examination No hemorrhages" Urine specimens submitted for examination during the months of August, September and October all showed the presence of a few red blood corpuscles, and the albumin varied between 15 and 2 gm per liter

Comment—In this patient the kidney changes may be assumed to be more recent and active, and consequently the added load of the high protein caused more lasting urinary disturbances than were seen in the preceding cases The albumin content of the urine increased markedly during the period of high protein feeding and decreased again upon return to the low protein diet Red blood corpuscles in the urine appeared promptly after high protein feeding was begun and were subsequently found at every examination The blood urea nitrogen was increased and remained at a relatively high level after several

TABLE 1

Case No	Blood Pressure		Pulse Pressure	2 Hr Phthalein		Average Protein Eaten, Gm per Kg per Day	Number Days Before R B C Found
	Systolic	Diastolic		Before High Protein	After High Protein		
1	230	150	100	20	22	2.7	21
2	200	115	85	35	75	2.8	1
3	165	95	70	24	20*	1.6	14
4	160	90	70	45	46	1.5	17
5	110	60	50	65	68	3.2	1

* Third day of high protein

days of low protein diet Following the high protein diet there was an increase in the engorgement and edema of the nerve head which diminished again after a long period of low protein diet The blood pressure was uninfluenced by high protein feeding

In two instances patients were studied in whom renal abnormalities were discovered by accident The blood pressure in each case was normal, and in one (Case 6) the urine examination gave no evidence of disease, while in the other there were a few red cells and occasional casts but no albumin

CASE 6—Male, aged 28, physician, married

Previous History—He had measles in early childhood and typhoid at 17 He has never had scarlet fever In August, 1919, he had a severe attack of tonsillitis at which time *Streptococcus hemolyticus* was isolated from his throat February, 1920, there was a second severe attack of tonsillitis, and in March, 1920, tonsillectomy was performed

Physical Examination—Physical examination showed nothing abnormal Blood pressure was 125/80 The urine specific gravity was 1.018 and there were no white or red blood cells, casts or albumin present Phenolsulphone-phthalein elimination was distinctly low, being 26 per cent for the first hour and 21 per cent for the second hour

Clinical Observation—October 1, high protein feeding was begun. The following morning as many as two red blood corpuscles were found per high power field, together with an occasional white blood cell and one granular cast. A questionable trace of albumin was present in the urine. On the following day, red cells were present in about the same numbers, and there was a distinct trace of albumin. After the second day, the high meat diet was discontinued, but red cells were still found in the urine on the following day, October 4. Subsequently, no red blood corpuscles and no casts were found at any of the urine examinations which were made daily until October 22. A trace of albumin was present until October 6. On the second day of the high meat diet, the phenolsulphonephthalein excretion was 16 per cent for the first hour and 22 per cent for the second hour, while the blood urea nitrogen was 15.8 mg per 100 cc. September 11 the phenolsulphonephthalein excretion was 19 per cent for the first hour and 20 per cent for the second hour, while September 22 it was 23 per cent in the first hour and 22 per cent in the second hour. An ophthalmoscopic examination after red cells appeared showed no abnormalities in the fundus.

Comment—Certainly the phthalein elimination in this subject was unusually low at each determination, but previous to the meat feeding there was no other indication of kidney abnormality. The blood chemistry was not done at this time. However, January 28, on his normal diet the total nitrogen of the blood was 35.4 mg, urea nitrogen, 17.3 mg, preformed creatinin, 1.8 mg, and uric acid, 4.75 mg per 100 cc. In view of the diminished phenolsulphonephthalein excretion in this case, together with the slightly increased blood uric acid, the conclusion is certainly justified that we are dealing with an early nephropathy. Promptly after forced meat diet, red cells and a trace of albumin appeared in the urine and phenolsulphonephthalein elimination, which was already low, fell from 26 to 16 per cent for the first hour.

CASE 7—Male, physician, aged 38. There was no knowledge of kidney disturbance of any kind and this case was studied as one of the normal control group reported in the second part of this paper.

Previous History—During infancy and childhood there were repeated attacks of croup and head colds. At the age of 7 and 14 tonsillectomies were performed, and at 21 he had an attack of double quinsy after which tonsillectomy was again done. In 1901, he had pneumonia with good recovery. In 1910, there was a severe antrum infection and a drainage was attempted by drilling into the sinuses. He has not had scarlet fever.

Physical Examination—The blood pressure was 135/90 and no evident abnormalities were present on physical examination.

The urine showed no albumin, but there was a trace of nucleo-albumin. There were a few hyalin and granular casts and from two to four red blood corpuscles were present in each high power field. Phenolsulphonephthalein elimination was 35 per cent for the first hour and 16 per cent for the second hour. Blood nitrogen and preformed creatinin were normal but the blood uric acid was slightly increased, being 4.6 mg per 100 cc.

Clinical Observation—Following these determinations large steaks were eaten at the noon and evening meals. A definite trace of albumin was present in the urine during the following day but was again absent during the next succeeding day. Red blood corpuscles and hyalin and granular casts were present at every examination. On the day following the meat meals, the phenolsulphonephthalein elimination was 48 per cent for the first hour and

14 per cent for the second hour as compared with the original determination of 35 per cent and 16 per cent for the first and second hours, respectively. The preformed creatinin of the blood remained practically unchanged, but the total nitrogen increased from 306 mg to 457 mg per 100 cc, and the uric acid from 46 to 51 mg per 100 cc. January 15, two weeks after the original examination, and again January 26, urine specimens were examined and found to be albumin free, but there were from two to four red blood corpuscles to a high power field and occasional granular and hyalin casts.

The feeding of high protein as a test for the presence of nephritis in cases of so called "essential hypertension" is inadequate to answer the original question, as will appear in Part 2, where it will be shown that it produced abnormalities in the urine of normal subjects. The work has, however brought to light information of great interest.

PART 2

RENAL IRRITATION IN NORMAL YOUNG MEN AFTER HIGH PROTEIN FEEDING

Since high protein feeding so regularly has been followed by the appearance of red blood corpuscles in the urine in the group of individuals described in Part 1, and since, in a few instances, a very short period of such feeding had this effect on kidneys which in the present state of our knowledge showed but slight evidence of damage, we asked ourselves the question whether even the normal kidney might not show evidence of irritation when forced to rid the body of the waste products resulting from a very brief period of protein excess.

As a preliminary experiment, one of us (T. S.) ate at 6 00 p. m. an enormous steak (about one pound) with a few potatoes and one cup of coffee. Urine passed at 8 00 p. m. was acid, specific gravity, 1.020, and free from albumin, white blood cells and casts, but contained from two to four red blood corpuscles per high power field. The blood urea nitrogen determined at 9 10 p. m. was 207 mg. Subjectively, there was a slight headache and the sensation of having merely over-eaten. During the twenty-four hours beginning at 8 00 a. m. the day following the excessive meat meal there was a total excretion of 13.39 gm. nitrogen in the urine. In the morning urine specimen there was no albumin, white blood cells or casts and no red blood cells could be found after a long and careful search. At 8 00 a. m. the blood urea nitrogen level was still slightly high—18.6 mg.

The effect of two high protein meals on normal men was next studied, medical students being used as subjects. On the day preceding the meat meals the total nitrogen, urea nitrogen, preformed creatinin and uric acid content of the blood were determined by the

TABLE 2—SHOWING THE EFFECT OF TWO FORCED MEAT MEALS IN NORMAL YOUNG MEN

Name	Day	Hour	Urine											Phthalein		Blood						
			Amount	Reaction	Specific Gravity	Albumin	Casts	Red Blood Corpuscles	White Blood Corpuscles	Total N.-trogen	Urea N.-trogen	Ammonia Nitrogen	Uric Acid	Creatinin	Chlorids	1st Hour	2d Hour	Total N.-trogen	Urea N.-trogen	Preformed Creatinin	Uric Acid	
L E H act 24	Before meat Meat day	5 00 p m 7 00 p m 11 00 p m Night	1 642	Ac Ac Ac	1 019 1 026 1 023	0 0 0	0 0 0	0 ++ ++	++ ++ ++	11 75	7 52	0 82	0 55	1 05	0 80	13 10	50	16	24 1	14 7	1 98	3 57
	After meat		1,729* 1,399	Ac Ac	1 019 1 025	0 0	0 0	0 0	++ ++	18 66* 21 96	12 38* 11 75	0 69* 0 87	0 73* 0 56	2 07* 2 02	0 93* 0 96	16 08* 13 40	62	?	58 2	32 4	2 23	2 52
SI act 31	Before meat Meat day	5 00 p m 11 00 p m Night	1,825	Ac Ac	1 023 1 027	0 0	0 0	0 ++	++ ++	11 09	5 03	0 40	0 32	1 50	0 85	15 5	47	10	33 9	15 6	1 74	2 30
	After meat		1,460* 1,540	Ac Ac	1 022 1 023	0 0	0 0	++ ++	++ ++	13 57* 27 45	7 00* 22 23	0 37* 0 52	0 33* 0 35	1 21* 2 07	0 57* 0 84	8 3* 12 9	47	13	48 9	21 4	2 01	4 02
Flz act 24	Before meat Meat day	5 00 p m 11 00 p m Night	1,810	N N N	1 019 1 020 1 018	0 0 0	0 0 0	0 ++ 0	++ ++ 0	8 56	5 54	0 34	0 20	1 63	0 45		52	16	22 9	13 0	1 66	1 87
	After meat		2,340* 2 005	N Ac	1 016 1 022	0 0	0 0	0 0	0 0	20 61* 19 64	14 32* 13 05	0 60* 0 50	0 38* 0 18	1 63* 2 30	0 44* 0 81		32	24	40 5	21 2	2 04	4 75
Oh act 30	Before meat Meat day	5 00 p m 11 00 p m Night	1,770	Ac N Ac	1 017 1 012 1 028	0 0 0	0 0 0	0 ++ ++	++ ++ ++	14 22	12 92	0 90	0 87	2 08	0 64	11 3	56	12	40 8	19 8	1 59	1 40
	After meat		2,280* 1,560	N Ac	1 014 1 021	0 0	0 0	++ ++	++ ++	22 11* 14 70	13 68* 10 20	0 82* 0 75	0 65* 0 45	2 31* 2 02	0 47* 0 36	10 2* 5 4	50	15	51 9	24 0	2 10	3 40

* Twenty four hour amount

Folin-Wu method Before the test the average amount of these various constituents was

Total N	30.4 mg per 100 c c
Urea N	15.7 mg per 100 c c
Preformed creatinin	1.74 mg per 100 c c
Uric acid	3.67 mg per c c

Phenolsulphonephthalein elimination averaged 48 per cent for the first hour and 14 per cent for the second hour. Quantitative determinations of the total nitrogen, urea nitrogen, ammonia nitrogen, uric acid, creatinin and chlorids of the urine were made in twenty-four hour periods preceding on the day of the meat meals and on the day following meat. These results are recorded in Table 2.

At noon and again at 6 00 p m of the meat day each subject ate a steak averaging about one and one-half pounds in weight. The urine passed between noon and 6 00 p m, that passed between 6 00 p m and 11 00 p m, and that passed between 11 00 p m and 8 00 p m was collected as separate specimens. Each specimen was tested for albumin and the sediment examined. Similar tests were made with the twenty-four hour urine preceding and following the meat diet. For quantitative determinations all the urine passed during the twenty-four hour period of the meat day was mixed.

At 8 00 a m on the day following the meat diet, blood analyses were made just as in the preliminary period. The average amounts found on this determination were

Total N	48.8 mg per 100 c c
Urea N	24.7 mg per 100 c c
Preformed creatinin	2.12 mg per 100 c c
Uric acid	3.67 mg per 100 c c

Consequently, on the morning following meat feeding there was an increase in the average total nitrogen, urea nitrogen preformed creatinin and uric acid of 63.8, 57.3, 21.8 and 59.5 per cent, respectively.

In no instance were red blood cells found in the urine preceding the meat meals. In every case red blood cells were present following the meat feeding. In no instance was albumin found at any examination.

It is evident, therefore, that protein excess for a period as short as one or two meals is sufficient to cause the appearance of red blood corpuscles in the urine of healthy young men.

DISCUSSION AND SUMMARY

Forced high protein feeding in four cases of hypertension in which urinary evidence of kidney disturbance was slight or absent resulted in each case in the appearance of red blood cells in the urine. On return to low protein diet, the red cells soon disappeared. In three cases, albumin was found after the meat diet, although previously none was

present In one case, albumin originally present as a trace was markedly increased following meat feeding In three of the four cases a definite increase in fundus changes was noted following the meat diet The one case of this group (Case 4) in which no increase in fundus changes was seen following meat presented originally the fewest signs of kidney abnormality It is interesting that in one instance following the added load of high protein diet there was a prompt albuminuria which quickly disappeared However, the addition of salt, which may be regarded as further increasing the work of the kidney, resulted in the reappearance of albumin followed shortly after by the appearance of red cells in the urine In one patient (Case 5) the history and urine findings pointed to a more recently active nephritis Albumin was present as a heavy cloud in the urine and there were a number of casts The blood pressure was normal After high protein feeding, red blood corpuscles which had previously been absent promptly appeared in the urine of this patient, and the amount of albumin was greatly increased Whereas, in all other cases the red blood cells disappeared promptly after a return to low protein diet, in this case red blood cells were found at every subsequent examination An increase in the engorgement and edema of the nerve head was also noted following the forced protein, while after a few days of low protein diet this edema and engorgement had distinctly decreased

Neither protein restriction nor high protein feeding had any influence on the blood pressure in any of these cases, and such a failure of protein diet to influence blood pressure agrees with the observation of Mosenthal⁵

Two cases of the series (Cases 6 and 7) were especially interesting inasmuch as previous to this study there had been no knowledge of kidney impairment In both cases preceding the test albumin was absent from the urine, while following the high protein a distinct trace was found, which again disappeared after return to normal diet In one of the cases (Case 7) inasmuch as red cells were found preceding the test, a slight increase in numbers would fail to give information of value, but in the other case (Case 6) red cells which were absent before the feeding were found after the high meat feeding and promptly disappeared on return to normal diet

Forced meat feeding for two successive meals was invariably sufficient to cause the appearance of red blood corpuscles in the urine of five normal young men On return to a normal diet, the red cells promptly disappeared In no case was there detectable albuminuria The nonprotein nitrogen constituents of the blood on the morning following the meat meals were much higher than before the test Pre-

⁵ Mosenthal H C Am I M Sc 160 808 1920

formed creatinin was increased least, being 21.8 per cent higher than on the day preceding the test, while urea nitrogen and uric acid were increased 57.3 and 59.5 per cent, respectively.

By consulting Table 2 it will be seen that Cases 5 and 2 with a daily protein intake of 3.2 and 2.8 gm per kilo, developed red cells in the urine on the second day, while in Case 1, although the daily protein ingested was 2.7 gm per kilo red cells did not appear for twenty-one days. It is suggestive that this last case showed the most evidence of kidney sclerosis of the series, the phenolsulphonephthalein elimination being lowest and the blood pressure and pulse pressure highest. It seems not improbable that the delay in the appearance of red cells here may be due, in part, at least, to a loss in flexibility of the kidney capillaries. It is worth while again calling attention to the fact that the phenolsulphonephthalein elimination for two hours increased in Case 2 from 35 per cent before meat feeding to 75 per cent after red cells had appeared, and in Case 5 from 65 to 72 per cent, while, on the other hand, in Case 1 the phenolsulphonephthalein for two hours was less than 20 per cent.

Assuming the truth of our assumption that high protein diet acts as a kidney irritant, it would be expected that in cases in the acute or sub-acute stage of nephritic involvement it would cause a more marked and persistent disturbance. That this assumption has support is evidenced by Case 5 of this series in which following meat the albumin output was greatly increased and the red cells persisted in the urine for three months during which time the patient was under observation, instead of promptly disappearing upon return to a normal diet. Further evidence pointing to an acute exacerbation of kidney disturbance in recently damaged kidneys after a high meat diet will be discussed in a subsequent paper.

1 Forced high protein feeding in the presence of kidney damage resulted in the appearance of red blood corpuscles in the urine, and in the appearance of albuminuria or in an increase of a pre-existent albuminuria.

2 Forced high protein feeding invariably resulted in the appearance of red blood corpuscles in the urine of normal men.

3 In some of the cases receiving high protein diet there was a definite increase in the edema and macular changes of the fundus following the meat.

4 High protein diet over a short period had no effect on the blood pressure.

CONCLUSION

Evidence is presented to show that high protein diet in man is a renal irritant.

II THE EFFECT OF CYANIDS AND OF ORGANIC OXIDIZING AGENTS ON THE LIVER INJURY CAUSED BY CHLOROFORM^{*}

N C DAVIS, MD†

SAN FRANCISCO

The series of experiments here recorded were undertaken with the hope of throwing some light on the etiology of chloroform liver necrosis. Should this liver injury be caused by a reduction of tissue oxidation, it is conceivable that the cyanids, which apparently act by preventing oxidation, might increase the damage to be expected from chloroform if the two drugs were given simultaneously. On the other hand, iodozo- and iodoxy-benzoic acids and their sodium salts, which readily give up oxygen to the tissues, might lessen the expected injury.¹

We have already reported a few experiments in which potassium cyanid was administered intravenously, either alone or in conjunction with prolonged chloroform anesthesia.² Although large doses of the drug itself produce an extensive fatty alteration in the liver parenchyma, smaller amounts, given during the administration of chloroform, affect the liver changes produced by the anesthetic very little, if at all.

Experimental work by Verworn³ tends to show that the depression caused by narcotics is really an asphyxiation. His hypothetical suggestion is that the oxygen carriers are unable to supply oxygen to the tissues, perhaps because the enzymes are of a lipoidal nature with consequent affinity for the usual anesthetics. Burge⁴ reports that the oxidative enzyme catalase is greatly reduced by anesthetics, most pronouncedly by chloroform. This might be considered to support the views of Verworn. However, both the method⁵ and the conclusions of Burge have frequently been questioned.⁶

* From the George Williams Hooper Foundation for Medical Research, University of California Medical School.

† University of California Fellowship.

1 We are indebted to Professor Loevenhart of the University of Wisconsin for liberal supplies of iodozo- and iodoxy-benzoic acids, also for valuable criticisms and suggestions relative to the experiments.

2 Davis N C, and Whipple, G H. *Arch Int Med* **23** 636 (May) 1919.

3 Verworn, Max. *Harvey Lectures* **7** 1911.

4 Burge, W E. *Am J Physiol* **43** 545, 1917, *Science* **46** 612, 1917, *J Pharmacol & Exper Therap* **12** 243, 1918, *ibid* **14** 121, 1919. Burge, W E, and Burge E L. *J Biol Chem* **41** 307, 1920. Burge, W E, Neill, A J, and Ashman R. *Am J Physiol* **45** 388 1918.

5 Bodansky, M J. *J Biol Chem* **40** 127, 1919.

6 Becht, F C. *Am J Physiol* **48** 171, 1919. Reimann, S P, and Becker C A. *J Physiol* **50** 54 1919. Stehle R L. *J Biol Chem* **39** 403, 1919.

Buckmaster ⁷ has shown that at the point of anesthesia with chloroform, from 64 to 71 per cent of the drug is held by the red corpuscles, after inhalation of 2 per cent chloroform for three-quarters of an hour, 98.5 per cent is found in the erythrocytes. He also demonstrated that in the first three minutes of anesthesia lung ventilation is reduced from 30 to 80 per cent, he considered, however, that the reduction of oxygen in the blood is not due to the latter cause but to the effect of the chloroform on the corpuscles (oxygen carriers).

Graham ⁸ has stated that chloroform is one of a group of substances whose effect on organs is like that of asphyxiation. He surmised that these drugs dissociate in the body, yielding a molecule with bivalent or unsaturated carbon which eagerly seizes oxygen. However, in a recent article ⁹ he has dismissed this theory. Buckmaster ⁷ has reported that he could not detect the formation of carbon monoxide from chloroform in the body.

Wells ¹⁰ has suggested that chloroform may first unite with the cell lipoids, perhaps physically, then poison the protoplasm by interfering with oxidative or synthetic functions.

The standard textbooks of pharmacology discuss the cyanids quite fully, Evans' ¹¹ article on "Cyanid Anoxemia" is a good recent contribution. Work seems to show that the cyanids prevent oxidations in the tissues themselves, whether by action on protoplasm directly or by inhibition or destruction of intermediary enzymes is not quite clear. The oxygen carrying power of the blood is not impaired.

Loevenhart and his co-workers have for many years been interested in physiologic oxidations. They have been able to produce central necrosis in rabbits' livers by suboxidation, the animals being left in an atmosphere of low oxygen tension for several days at a time ¹². Loevenhart and Grove, ¹³ also Arkin ¹⁴ have studied the preparation of iodoxy-

7 Buckmaster, G. A. Proc Roy Soc Med, Lond, Sect Anesth **11** 15, 1918

8 Graham, E. A. J Exper M **15** 307 1912, J A M A **69** 1666 (Nov 17) 1917

9 Graham, E. A. Arch Int Med **25** 575 (June) 1920

10 Wells, H. G. J A M A **46** 341 (Jan 29) 1906, Arch Int Med **1** 589 (April) 1908, Chemical Pathology, Philadelphia, W. B. Saunders Co., 1918, Ed. 3

11 Evans, C. L. J Physiol **53** 17, 1919

12 Martin, G. H., Bunting, C. H., and Loevenhart, A. S. J Pharmacol & Exper Therap **8** 112, 1916

13 Loevenhart, A. S., and Grove, W. E. J Pharmacol & Exper Therap **1** 289, 1909

14 Arkin, A. J Infect Dis **13** 408, 1913

15 Amberg, S., and Knox, J. H. M. J Pharmacol & Exper Therap **3** 223, 1911. Amberg, S. Ztschr f d ges exper med **2** 19, 1913. Amberg, S., Loevenhart, A. S., and McClure, W. B. J Pharmacol & Exper Therap **10** 209, 1917

16 Loevenhart, A. S., and Grove, W. E. J Pharmacol & Exper Therap **3** 101, 1911. Grove, W. E., and Loevenhart, A. S. J Pharmacol & Exper Therap **3** 131, 1911

benzoic and iodozo-benzoic acids Amberg,¹⁵ Loevenhart,¹⁶ Arkin,¹⁷ and others have studied the effect of these acids and their sodium salts on inflammatory reactions, and the antagonism to cyanids. It has been found that cyanids intensify and organic oxidizing agents diminish inflammatory processes, such as the mustard oil reaction and the intracutaneous reaction in serum sensitization, these results seem to be due to the effect on bodily oxidations and reductions. Arkin¹⁸ tested the bactericidal property of sodium iodoxy-benzoate and found a definite relationship between this and its oxidizing power. Arkin and Fink¹⁹ also studied the effect of this sodium salt on catalase in the blood and tissues, they found that catalase normally varies immensely and that the values after administration of iodoxy-benzoate fell within normal limits. Loevenhart and associates²⁰ have recommended the therapeutic use of sodium cyanid as a respiratory stimulant. Loevenhart and Eyster²¹ found that iodozo- and iodoxy-benzoates were unable to replace molecular oxygen for the maintenance of activity in the isolated mammalian heart. These oxidizing agents were reduced, however, and caused the heart to develop rigor, probably because of the oxidation of some substance in its musculature. Iodozo- and iodoxy-benzoates apparently transfer their available oxygen directly to the substance to be oxidized, without an intermediate liberation of free oxygen, certain oxidizing enzymes seem to facilitate the transfer, in certain peroxidase reactions the organic oxidizing agents act in a manner comparable to hydrogen peroxid.¹⁰

METHODS

Dogs have been used as experimental animals. Rats were tried, but even the controls differed so much in reaction that the experiments are of little value, these individual variations were probably due, in part, to nutritional differences and the difficulty of imposing a fasting period to reduce the animals to a starvation base line, moreover, the immediate mortality from chloroform injection was very high. Dogs have always been starved three or four days to eliminate the effect of diet on the chloroform injury.

Squibbs' chloroform has been used for both anesthesia and injections. Chloroform anesthesia has been light, the drug was administered by the drop method. When given subcutaneously, chloroform was mixed with twice its volume of liquid petrolatum, syringe plungers

17 Arkin, A. J. Infect Dis **16** 349, 1915

18 Arkin, A. J. Pharmacol & Exper Therap **3** 145, 1911

19 Arkin, A., and Fink, E. B. J. Infect Dis **22** 515, 1918

20 Loevenhart, A. S., Lorenz, W. F., Martin, H. G., and Malone, J. Y. Arch Int Med **21** 109 (Jan) 1918

21 Loevenhart, A. S., and Eyster, J. A. E. J. Pharmacol & Exper Therap **5** 21, 1913

were removed and the substances measured directly into the barrels, first, the oil then the chloroform from a 1 c c pipet calibrated to hundredths, the mixtures were injected immediately after measurement. Dogs were under light ether anesthesia during the injection of chloroform and other substances. The amount of chloroform injected has not been of itself sufficient to produce unconsciousness, or even instability of equilibrium in many cases.

A liver injury produced by either method may be repeated accurately under the same experimental conditions if an intervening recovery period of three or four weeks is allowed. This possibility has been discussed in a previous communication²² and subsequent experiments have verified our belief.

Specimens of iodozo- and iodoxy-benzoic acids were furnished by Dr. Loevenhart. His directions for preparing solutions for intravenous injections were briefly: Dissolve 1.32 gm iodozo-benzoic acid, or 1.4 gm iodoxy-benzoic acid, in 20 c c water and 10 c c normal sodium hydroxid and dilute to 100 c c with water; solutions are to be prepared fresh just before using (intravenously). We never had occasion to use 100 c c at one time, hence we made up smaller amounts proportionately and by similar steps. To facilitate giving over an extended period of anesthesia these amounts were further diluted with physiologic sodium chlorid solution. When given subcutaneously, iodoxy-benzoic acid was used in relatively large amounts in small volumes of alkaline fluid. Since sodium hydroxid was the solvent, the drug actually employed was sodium iodoxy-benzoate or iodozo-benzoate, as the case might be, however, in the tables and protocols the record gives only the amount of acid corresponding to the volume of solution used. For instance, if 0.2 gm acid was dissolved in 200 c c alkaline fluid, and 150 c c of this solution was used, the records show 0.15 gm acid administered. Iodoxy-benzoate solution given subcutaneously was diluted with 10 per cent acacia solution, iodozo-benzoic acid was given subcutaneously as a suspension in 10 per cent acacia solution.

The potassium cyanid employed was a Braun-Knecht-Heimann product, from 98 to 99 per cent pure; the sodium cyanid was supposed to be chemically pure. The cyanids were dissolved in physiologic sodium chlorid solution immediately before use.

Large amounts of fluid given intravenously have been administered by gravity method, from a funnel through a rubber tube with a glass section near the vein to act as a window, into the external jugular vein through an ordinary large syringe needle; rapidity of flow has been controlled with a screw pinch-cock.

22 Davis, N. C., and Whipple, G. H. *Arch. Int. Med.* **23**:612 (May) 1919.

In case the dogs survived, it has been our routine to operate two days after the administration of chloroform and remove a small piece of liver, either by ligating and snipping off the tip of a pointed lobe, or by inserting a mattress suture in the edge of a larger lobe and excising a wedge of tissue from the area thus deprived of blood. Operations have been performed always under ether anesthesia, and with aseptic precautions. We consider that the best time to estimate tissue injury is on the second day following the injection of the chloroform, since the necrosis is then usually well demarcated and debris is not yet in process of removal. Specimens are fixed in 10 per cent formaldehyde solution and divided for (1) frozen sections (fat stains, etc.), and (2) paraffin embedding with subsequent hematoxylin and eosin staining. Estimates of necrosis and fatty degeneration are always based on microscopic examination of the paraffin sections.

The fact that we consider one small specimen of liver tissue as representative of the whole may appear unwarranted. Dowler and Mottram²³ studied the fat content of different parts of the liver after injection of fat into the hepatic artery, glycogen after a heavy meal of cane sugar, and fatty acids during fasting periods, and concluded that the distribution of these substances is irregular and unpredictable, however, they admit that the liver of a fasting dog gives the nearest approach to even distribution. Sérége²⁴ in determining urea and glycogen, and Bartlett, Corper and Long²⁵ in studying the distribution in the liver of fat injected in the portal vein found considerable differences between the lobes as a whole, but intralobular uniformity. Differences of distribution of these various substances have been correlated with differences in blood supply, i.e., the blood from various portal tributaries does not thoroughly mix, hence is distributed differently. On the other hand, Brissaud and Bauer,²⁶ also Gilbert and Villaret²⁷ found an equal distribution of injected gelatin throughout the liver.

When dogs have died of chloroform liver injury we have always taken sections from various parts of the liver, representing several lobes. Ignoring the single layer of liver lobules immediately beneath the capsule (which are usually severely injured), these sections have invariably shown the same picture. Consequently, we have always felt justified in basing our estimations on a biopsy specimen from one lobe only.

²³ Dowler, V. B., and Mottram, V. H. *J. Physiol.* **52** 166, 1918.

²⁴ Serege. *Compt. rend. Soc. d. Biol.* **54** 200, 1902, **56** 597, 600 1904, **58** 519, 1905. (Cited by Dowler.)

²⁵ Bartlett, F. K., Corper, H. J., and Long, E. R. *Am. J. Physiol.* **35** 36, 1914.

²⁶ Brissaud and Bauer. *J. d. l'Anat.* **45** 1, 1909.

²⁷ Gilbert, A., and Villaret, M. *Arch. d. med. exper.* **21** 373, 1909.

EXPERIMENTAL OBSERVATIONS

Table 1 summarizes the essential details of experiments in which organic oxidizing agents were given intravenously during chloroform anesthesia. A representative protocol follows, it is scarcely necessary to add protocols for all the dogs.

EXPERIMENT 2 — *Control Simple Starvation and Chloroform Anesthesia*
Dog 19-39, female Airdale

Oct 8, 1918 Weight, 261 pounds Isolated before daily feeding, in good condition Fasting begun

October 9 Weight, 258 pounds, October 10 weight 248 pounds No food

October 11 Weight, 245 pounds Active and healthy Chloroform anesthesia for one and a quarter hours (from 5 50 to 7 05 p m)

October 12 Weight, 231 pounds Bright and active

October 13 Weight, 229 pounds Bright and active

October 14 Weight, 228 pounds Piece of liver removed at 9 30 a m Sections show approximately 60 per cent necrosis (beginning resolution), fat (scattered) Mixed diet

Chloroform Anesthesia Plus Sodium Iodoxy-Benzoate Intravenously —
Dog 19-39, female Airdale

March 5, 1919 Weight, 338 pounds Isolated before daily feeding, fasting begun, has completely recovered from previous experiments, in excellent condition

March 6 and 7 No food

March 8 Weight, 315 pounds Active and healthy Chloroform anesthesia for one and a quarter hours (from 2 45 to 4 p m) Intermittently during this period 0.21 gm iodoxy-benzoic acid (freshly prepared in 15 cc alkaline solution, then diluted to 200 cc with physiologic sodium chloride solution) was given intravenously. No ill effects of moment were observed either during or following the injection.

March 9 Active Mixed food

March 10 Active Piece of liver removed at 1 30 p m Considerable bleeding occurred Milk and mixed diet Sections show approximately 60 per cent necrosis with a scattering of fat in the dead tissue

These experiments seem to indicate that iodoxy- and iodozo-benzoic acids have little, if any, protective value against liver necrosis when given intravenously. This may be due to the small amounts of the drugs so given. The intravenous solutions were administered slowly and fairly uniformly throughout anesthesia, intermittently (as in Experiment 2), or steadily during the first forty-five or sixty minutes of anesthesia and none the last few minutes. In Experiment 1 some difficulty was encountered due to apnea during anesthesia, but the dog roused somewhat afterward, death, probably from the iodoxy-benzoate, occurred too early to determine accurately the extent of liver injury, there seemed to be some central disintegration, however. In Experiment 4 there appeared to be a definite lessening of necrosis after injection of iodozo-benzoate, in this case intravenous injection was continued steadily during the first hour of anesthesia, the liver showed one-quarter to one-third necrosis with extensive vacuolization and fat up to one-half, the uninjured lobule peripheries showed considerable

TABLE 1—CHLOROFORM INTALATION PLUS ORGANIC OXIDIZING AGENTS INTRAVENOUSLY

Experiment Number	Dog Number	Date, 1919	Weight, Lbs	Fasting Period, Days	Chloroform Anesthesia, Hours	Organic Oxidizing Agent	Liver Injury		Remarks
							Oxidizer Plus Chloroform	Control Chloroform	
1	1930	Feb 12	18.6	4	1 1/4	0.21 gm iodoxy benzoic acid	?	60% necrosis	Found dead next morning, liver shows some central disintegration, necrosis not determinable, viscera congested
2	1939	Mar 8	21.5	1	1 1/4	0.21 gm iodoxy benzoic acid	60% necrosis	60% necrosis	
3	1971 (a)	Feb 25	13.6	4	1 1/4	0.14 gm iodoxy-benzolc acid	50% necrosis	50% necrosis	
4	1971 (b)	Apr 16	13.8	1	1 1/4	0.25 gm iodozo benzoic acid	25% necrosis	50% necrosis	Glycogen appears in liver lobule peripheries, suggesting dog fed by mistake during starvation period
5	1928	May 6	19.1	4	1 1/4	0.24 gm iodozo benzoic acid	50% necrosis	50% necrosis	
6	1915	May 24	46.5	4	1 1/4	0.35 gm iodozo benzoic acid	60% necrosis		Operation two days following chloroform, found dead on sixth day following operation, intense jaundice, hemorrhages in viscera, slight removal of liver debris

glycogen This glycogen content leads to the question whether or not the dog received food by mistake during the preliminary period of supposed fasting This storage might have occurred after chloroforming and before operation, but more likely it took place before the administration of chloroform Glycogen per se may have no protective value against chloroform, but its presence is certainly somewhat of a gage of carbohydrate assimilation, which for some reason is protective A smaller amount of iodoxy-benzoate had no protective value for this same dog (Experiment 3) Unfortunately, there is no control on the same animal in Experiment 6, but judging from the injuries sustained by the other dogs recorded in the same table, there appears to have been no protection from the amount of iodoxy-benzoic acid given

To summarize Table 1, there seems to have been no protection against chloroform injury from intravenous administration of iodoxy- and iodoxy-benzoates in five of six experiments, there seems to have been protection in one case from iodoxy-benzoate, but this protection may very well have been from food given by mistake, and not from the drug

Table 2 summarizes briefly the experiments in which chloroform was given subcutaneously with or without organic oxidizing agents and more ample summaries of certain experiments follow

EXPERIMENT 13—Dog 20-17, young black mongrel, female

Sept 26, 1919 Weight 189 pounds Isolated before daily feeding, healthy and active, fasting begun

September 27 and 28 No food

September 29 Weight, 172 pounds (78 kilos) Active Gave subcutaneously 117 cc chloroform (0.15 cc per kilo) plus 24 cc liquid petrolatum, temporary ether anesthesia

September 30 Bright and active

October 1 Weight 165 pounds Removed piece of liver at 2 p m Sections show about 35 per cent necrosis with severe vacuolation and injury up to one-half, fat moderate Recovery on mixed diet

EXPERIMENT 21—Dog 20-17, young black mongrel female

October 21 Weight, 20 pounds In good condition and active, isolated after daily feeding, fasting begun

October 22, 23 and 24 No food

October 25 Weight, 18 pounds (8.18 kilos) Active Gave subcutaneously 122 cc chloroform (0.15 cc per kilo) plus twice its volume liquid petrolatum, followed immediately by 1 gm iodoxy-benzoic acid dissolved in 7 cc tenth-normal sodium hydroxide plus 10 cc of a 10 per cent acacia solution subcutaneously Temporary ether anesthesia

October 26 Bright and active

October 27 Active Piece of liver removed at 1 p m Hard swelling in left lumbar quadrant at point of injection of iodoxy-benzoate Sections show from 10 to 15 per cent necrosis, very slight amount of fat over central half of lobules Recovery on mixed diet Slough at point of injection

As will be noted from Table 2, massive doses of the organic oxidizing agents given subcutaneously apparently have protective value against chloroform injury, in some cases this is quite striking Iodoxy-benzoic

TABLE 2—COMPARATIVE TABLE—SUBCUTANEOUS INJECTIONS

Chloroform Alone						Chloroform and Oxidizing Agent						
Experiment Number	Dog Number	Fasting Period, Days	Chloroform Cc per Kilo (subcutaneous)	Liver Injury	Remarks	Experiment Number	Dog Number	Fasting Period, Days	Chloroform Cc per Kilo (subcutaneous)	Organic Oxidizing Agent (subcutaneous)	Liver Injury	Remarks
7	20-7	1	1.00	75% necrosis	Found dead on second day	19	20-38	4	0.20	Iodoxy benzoic acid 0.6 gm	50-60% necrosis	Found dead on seventh day apparently from toxemia, injection slough, liver repairing well
8	20-10	1	0.50	80% necrosis	Died on second day							
9	20-12	1	0.20	80% necrosis	Found dead on third day	20	20-65	1	0.22	Iodoxy benzoic acid 0.5 gm	60% (+) necrosis	Operation on second day found dead on fourth day debris in liver largely removed, dog developed distemper during experiment
10	20-13	2	0.10	No necrosis trace of fat	Minimal injury							
11	20-13	3	0.20	75% necrosis	Found dead on third day	21	20-17	4	0.15	Iodoxy benzoic acid 1 gm	10-15% necrosis, slightest possible trace of fat	Recovered
12	20-18	1	0.16	70% necrosis	Found dead on next day							
13	20-17	4	0.15	35% necrosis, severe injury	Recovered, see experiment 21 on same dog	22	20-57	3	0.21	Iodoxy benzoic acid 0.8 gm	No necrosis, no fat	Liver sections from operation show glycogen, died on seventeenth day from pneumonia injection slough
14	20-75	3	0.15	50% (+) necrosis	Operation on second day, found dead on fourth day	23	20-76	3	0.21	Iodoxy benzoic acid 0.8 gm	45% necrosis, very severely injured up to 1/2	Recovered, very slight injury from injection
15	20-76	3	0.21	60% necrosis	Recovered, see experiment 23 on same dog, second control on same dog shows only about 50% necrosis							
16	20-31	4	0.18	55% necrosis	Found dead on second day, see experiment 24 on same dog	24	20-31	4	0.18	Iodoxy benzoic acid 0.5 gm	Slightest possible trace necrosis, trace fat over 1/2	Recovered, injection slough
17	20-30	4	0.14	60% necrosis	Recovered, same result obtained on two occasions, once before and once after experiment 25							
18	20-106	4	0.14	85% necrosis	Died on second day	25	20-30	4	0.14	Iodoxy benzoic acid 0.5 gm	45% necrosis	Recovered, injection slough

acid was dissolved in tenth normal sodium hydroxid about 0.1 gm to 1 c c, and the volume increased by addition of 10 per cent acacia solution, no doubt the ugly sloughs obtained in such instances were due to the alkalinity of the solution (unfortunately much stronger than necessary), not to the drugs used, controls where alkaline solutions alone were used showed the same sloughs. Iodozo-benzoic acid was given suspended in 10 per cent acacia solution, these injections did not cause such severe reactions, but the drug seemed to be absorbed only partially. With the exception of the sloughs already mentioned there have been no ill effects noted from injections of either drugs. On one occasion a large dose of iodozo-benzoic acid suspension was given to a dog intraperitoneally, this caused an acute reaction and contributed largely to the dog's death within a few hours (chloroform was given also, subcutaneously). Intraperitoneal injections of from 10 to 20 mg iodozo-benzoic acid in rats sometimes seem very toxic, in other cases apparently cause no reaction.

It is interesting to note the very small doses of chloroform which may prove fatal when given subcutaneously to dogs after a preliminary fast. Experiments 10 and 11 indicate the narrow zone of safety between a dose producing no injury and that causing death.

Experiment 22 indicates superficially a very marked protection, but since the liver showed glycogen at operation, it may be a food protection, either because of an inadequate starvation period, or because of feeding by mistake on fasting days.

Table 3 merely amplifies experiments recorded in Table 2 and adds Experiments 26 and 27 to complete the record of Dog 20-30. Experiment 26 will be taken up later in discussing the use of cyanids. Experiment 27 is merely a repetition of 17, to act as double control. This double check was attempted on Dog 20-76 (Table 2), but the second trial with chloroform alone showed only about 50 per cent necrosis, compared with the original 60 per cent, this is the only time that two controls on the same animal have failed to check, and this is not a very wide fluctuation considering that estimations are merely relative.

It will be noted that Experiment 17 was intended as a control not only for Experiment 25 on the same animal, but for Experiment 16 on Dog 20-31, while Experiment 24 controls Experiment 16 on the same animal. A point worth noting is that the weights of these dogs in control experiments were less than in the others, in such cases the animals actually received less chloroform, but in the cases with greater weights the surplus fat may have been more than sufficient as a block to chloroform to compensate for the greater injection, ignoring any possible effect from organic oxidizing agents. However, no such

TABLE 3—EFFECT OF IODOXY-BENZOATE

	Experiment Number	Date of Injection	Fasting Period, Days	Weight at Time of Injection, Kilos	Chloroform, O c per kilo (subcutaneously)	Potassium Cyanid (subcutaneously), Mg	Iodoxy benzoic Acid (subcutaneously), Gm	Amount of Liver Injury	Remarks
Dog 20 30 Small male terrier	17	11/10/19	4	5.80	0.14			60% necrosis, slightest possible trace fat	Questionable distemper during convalescence, wound healed slowly
	25	12/18/19	4	6.42	0.14		0.5	45% necrosis, slight trace fat	Iodoxy benzoic acid dissolved in alkaline sodium solution, injection slough
	26	2/4/20	4	6.30	0.14	25		45-50% necrosis, slight trace fat	Potassium cyanid dissolved in 12 cc physiological sodium chloride solution
	27	3/22/20	4	6.02	0.14			60% necrosis, slight trace fat	Experiment performed as second control
Dog 20 31 Young female collie	16	11/10/19	4	6.60	0.18		0.5	Slightest possible necrosis trace of fat over 2% of each lobule	Iodoxy benzoic acid dissolved in alkaline sodium solution, questionable distemper during convalescence injection slough
	24	12/18/19	4	5.68	0.18			55% necrosis, slightest possible trace fat	Alkaline sodium solution injected, found dead on second day necrosis at point of injection, edema and congestion in lungs

explanation will hold in the case of Dog 20-76, for here the weights in control experiments were practically the same, and both slightly greater than the weights in experiments when other substances were injected, in which latter cases the injuries sustained were less

The tables and protocols which follow present a few experiments in which cyanid were used in conjunction with chloroform

EXPERIMENT 28—*Control, Chloroform Anesthesia* Dog 19-74, young black and white male terrier

Dec 7, 1918 Weight, 118 pounds In good condition, fourth day of fasting, chloroform anesthesia for one and a quarter hours

December 9 Weight, 111 pounds Active Piece of liver removed in afternoon, bled freely Sections show necrosis involving one-half of each liver lobule, and a moderate degree of fatty degeneration in zone surrounding necrosis

Chloroform Anesthesia, Cyanid Subcutaneously Dog 19-74, a black and white male terrier

Oct 18, 1919 Weight, 15 pounds In good condition, isolated before daily feeding, fasting begun Since the control experiment (given above) was performed, this animal has been subjected to two experiments in which organic oxidizing agents were injected intravenously (Table 1), also a second control with result the same as in the first

October 19 and 20 No food

October 21 Weight, 139 pounds Active Chloroform anesthesia for one and a quarter hours Gave 15 mg potassium cyanid in 30 cc physiologic sodium chlorid solution subcutaneously in divided doses at intervals during anesthesia

October 23 Weight, 133 pounds Active Piece of liver removed at 1 30 p m, many adhesions, considerable bleeding, some subcutaneous edema in area of injections (due to alkalinity of potassium cyanid) Sections show approximately 60 per cent necrosis

EXPERIMENT 15—*Control, Chloroform Subcutaneously* Dog 20-76, a young, black mongrel, female

Jan 24, 1920 Weight, 142 pounds Active and in good condition Isolated before daily feeding, fasting begun

January 25 No food

January 26 (Third day of fasting) Weight, 129 pounds (5.85 kilos) At 5 p m gave 123 cc chloroform (0.21 cc per kilo) plus 2.5 cc liquid petrolatum subcutaneously, temporary ether anesthesia

January 27 Weight, 124 pounds Quite active

January 28 Weight, 123 pounds Dog in fair condition Piece of liver removed at 3 30 p m Sections show 60 per cent necrosis, fat plus

EXPERIMENT 30—*Chloroform Subcutaneously, Cyanid Subcutaneously* Dog 20-76, a young, black mongrel female

March 19 Weight, 148 pounds In excellent condition Isolated after daily feeding, fasting begun

March 22 (Third day of fasting) Weight, 129 pounds (5.85 kilos) Gave 123 cc chloroform (0.21 cc per kilo) plus 2.5 cc liquid petrolatum subcutaneously, followed immediately by 35 mg potassium cyanid in 35 cc physiologic sodium chlorid solution subcutaneously, temporary ether anesthesia

March 23 Active Full diet

March 24 Active Piece of liver removed at 3 15 p m Sections show from 35 to 40 per cent necrosis Point of injection of potassium cyanid later showed a little local necrosis (due to alkalinity of potassium cyanid)

TABLE 4—POTASSIUM CYANID AS INFLUENCING CHLOROFORM LIVER INJURY

Experiment Number	Dog Number	Weight, Kilos	Fasting Period, Days	Chloroform	Potassium Cyanid (subcutaneous), Mg	Liver Injury	Control Liver Injury (Same Dog)	Organic Oxidizing Agent Injection (Same Dog)	Remarks
28	1974	6.3	4	1 1/4 hours anesthesia	15	60% necrosis	50% necrosis (chloroform anesthesia, no drugs—2 controls with same result)	50% necrosis (chloroform anesthesia, 0.14 gm iodoxy benzoic acid intravenously) 25% necrosis (chloroform anesthesia, 0.25 gm iodoxy benzoic acid intravenously with question of feeding during stimulation period)	Greater injury caused by potassium cyanid
29	2017	8.6	1	0.13 c c per kilo subcutaneously	20	35-40% necrosis	35% necrosis (very severe injury up to 1/2)	10-15% necrosis (1 gm iodoxy benzoic acid subcutaneously)	A shade more necrosis in potassium cyanid experiment, but not so much surrounding injury
26	2030	6.3	4	0.14 c c per kilo subcutaneously	25	45-50% necrosis	60% necrosis (2 controls with same result)	15% necrosis (0.5 gm iodoxy benzoic acid subcutaneously)	Less necrosis in potassium cyanid experiment than in controls about the same as with organic oxidizing agent injection
30	2076	5.9	3	0.21 c c per kilo subcutaneously	35	35-40% necrosis	(1) 60% necrosis (2) 50% necrosis	45% necrosis (0.8 gm iodoxy benzoic acid subcutaneously)	Less necrosis in potassium cyanid experiment than in control or when using iodoxy benzoic acid

Between Experiments 15 and 30 came Experiment 23 in which the dog was given iodozo-benzoic acid subcutaneously (Table 2) In May this dog was used for another control similar to Experiment 15, and showed approximately 50 per cent necrosis In a final experiment in June the dog was given 26 mg sodium cyanid (molecular equivalent of the 35 mg potassium cyanid in Experiment 30), but 12 mg of this were injected intraperitoneally and death occurred from respiratory failure in about one hour

EXPERIMENT 35—*Chloroform Anesthesia, Sodium Cyanid Intravenously* Dog 21-18, a brindle mongrel, male, right eye shows keratitis and staphyloma, suggesting previous distemper infection

Sept 14 1920 Weight, 25.5 pounds In good condition and active Isolated after daily feeding, fasting begun

September 15, 16, 17 No food

September 18 Weight, 23.8 pounds Active Chloroform anesthesia for forty-five minutes (3 to 3.45 p. m.) During thirty-five minutes of anesthesia 10 mg sodium cyanid in 100 c.c. physiologic sodium chlorid solution were given intravenously (0.01 per cent solution = approximately 1/500 N) Considerable chloroform was administered, but some respiratory difficulty was encountered because of sodium cyanid More than the 100 c.c. would have been given except for shallow breathing, tendency to apnea, and a rapid, weak pulse

September 19 Weight, 23.3 pounds A little dull Casein feeding

September 20 Weight, 23.5 pounds Somewhat dull Casein feeding Operation at 3 p. m. Piece of liver removed, liver is grossly injured Sections show about 35 per cent necrosis and a trace of fat

EXPERIMENT 36—*Chloroform Anesthesia, Physiologic Sodium Chlorid Solution Intravenously* Dog 21-18 A brindle, mongrel male

Oct 19, 1920 Weight, 30.7 pounds Bright and active Wounds of former operations healed Isolated after daily feeding, fasting begun

October 20, 21 and 22 No food

October 23 Weight, 27.5 pounds Active and in good condition Chloroform anesthesia for forty-five minutes (3.40 to 4.25 p. m.) Gave 100 c.c. physiologic sodium chlorid solution intravenously during anesthesia

October 24 Weight, 27 pounds Quite active Gelatin feeding

October 25 Weight, 25.5 pounds Active No vomiting Operation at 2.30 p. m. Piece of liver removed, liver appears injured in gross Sections show approximately 55 per cent necrosis and a moderate amount of fat

The experiments in which potassium cyanid was given subcutaneously appear very irregular and contradictory On account of the immediate toxicity, the drug was given cautiously in rather small dosage, however, this accounts in no way for the fact that some cases show even less injury than the control experiments on the same animals, it may be coincidence that the larger doses of potassium cyanid (25 and 35 mg) seem to have been somewhat protective It will be recalled that we found no effect on chloroform liver injury from small intravenous injections of potassium cyanid

Since in one case a small area of necrosis appeared at the point of injection of potassium cyanid, and in other cases moderate tissue edema

TABLE 5—SODIUM CYANID INTRAVENOUSLY AS INFLUENCING CHLOROFORM LIVER INJURY

Experiment Number	Dog Number	Weight, Pounds	Fasting Period, Days	Chloroform Anesthesia, Min	Sodium Cyanid Intravenously, Mg	Liver Injury	Remarks
31	20 108	17.5	4	45		60% necrosis	(control) Recovery operation on second day following anesthesia
32	20 112	14.8	4	45		70% necrosis	Control Found dead on second day following anesthesia
33	20 119	28.0	4	45	18	60% (+) necrosis, fat very extensive almost to lobule peripheries	Sodium cyanid in physiologic sodium chlorid solution, 1 mg to 10 cc intervals of accelerated breathing during anesthesia, periods of apnea the last few minutes, recovery, operation on second day following chloroform
34	20-119	27.4	4	45		75% (+) necrosis fat moderate	Control (see Experiment 33) Found dead on second day following anesthesia
35	21 18	23.8	4	45	10	35% necrosis trace of fat	Sodium cyanid in physiologic sodium chlorid solution 1 mg to 10 cc tendency to apnea and rapid, weak pulse, especially toward the last, recovery, operation on second day
36	21-18	27.5	4	45		55% necrosis, fat moderate	Control (see Experiment 35) 100 cc physiologic sodium chlorid solution intravenously during anesthesia, recovery operation on second day

was manifested, we must conclude that a considerable local reaction took place (due in part to alkalinity of the drug), and it seems likely that not only the tissue suffered but that a certain part of the potassium cyanid may have been destroyed locally

Table 5 gives the results of sodium cyanid injections in two dogs, these same animals were later used as controls, two other control experiments on different animals are also included. These experiments indicate that the cyanid may have some protective value against chloroform injury. The attempt was made to give the anesthetic at the same rate in all cases, but on account of respiratory difficulties caused by the cyanid it is possible that the animals receiving such injections were given somewhat less chloroform. This is suggested as a possibility only, but if so might account for the lessened liver necrosis in such cases. For comparative purposes we consider that controls on the same animals are by far the most valuable.

DISCUSSION

Although these experiments were undertaken to throw some light on the rôle of oxidations in chloroform liver injury, the results are far from conclusive. There appears to have been a definite amount of protection in the cases when organic oxidizing agents were injected in large amounts subcutaneously. Should this have been because an increased supply of oxygen was furnished to a liver hampered by its lack, we should have expected cyanids to have acted in an opposite manner, as found by Loevenhart, Amberg and their associates in various conditions, as a matter of fact, relatively large doses of cyanids seem to have been themselves rather protective in certain instances.

Administration of cyanids intravenously during chloroform anesthesia has been difficult because of respiratory irregularities, this trouble has been much more marked than in control experiments (without the cyanid). Loevenhart has had less difficulty in giving a solution of sodium cyanid five times as concentrated as that used by us. There are two factors in our experiments which may account, in part, for this high immediate cyanid toxicity: (1) preceding fasting period, and (2) simultaneous chloroform anesthesia.

We are safe in saying that the experiments do not prove that liver necrosis following administration of chloroform has any connection with a disturbance of normal tissue oxidation.

At the present state of our knowledge it is hardly justifiable to assume that chloroform narcosis and chloroform liver necrosis are manifestations of the same pharmacologic property of the drug, this point is often left rather indefinite in the literature on the subject. It is relatively simple to obtain one without the other. Since our observa-

tions relate entirely to the phenomenon of liver injury, we do not extend our deductions to bear at all upon the existing theories pertaining to the anesthetic action of chloroform

SUMMARY

Very small amounts of chloroform given subcutaneously are sufficient to cause an extensive liver injury in fasting animals

The intravenous injection of small amounts of organic oxidizing agents (iodoxy- and iodozo-benzoic acids and their sodium salts) seems to have no effect on chloroform liver necrosis. Large amounts of these substances subcutaneously appear to afford the liver some protection against chloroform injury

The administration of cyanids (potassium and sodium) does not effect chloroform liver injury in any constant manner, it seems that large doses may exert some protective action

These experiments offer no evidence that chloroform liver injury is a result of disturbance in tissue oxidations

ON THE ETIOLOGY OF AN OUTBREAK OF INFECTIOUS DIARRHEA^{*}

HARRY WEISS

BOSTON

In the late summer and autumn of 1918, an outbreak of infectious diarrhea occurred at the St Anne Barracks, Nantes, in Base Section No 1, A E F. In the course of an investigation of this outbreak, I isolated a micrococcus from a number of cases which shows evidence of being the etiologic agent.

Infectious diarrhea had been prevalent throughout the base section, especially in the camps in the vicinity of St Nazaire, and epidemics of a similar nature had been reported in other parts of France. The prevalence of infectious diarrhea throughout the A E F was known to practically all the members of it, and particularly to those who were directly concerned with the control of the communicable diseases. There are no data available giving the exact number of cases that occurred. Repeated attempts to obtain information resulted only in approximations based on the total strength of the command. These estimates varied from an incidence of 25 to 100 per cent.

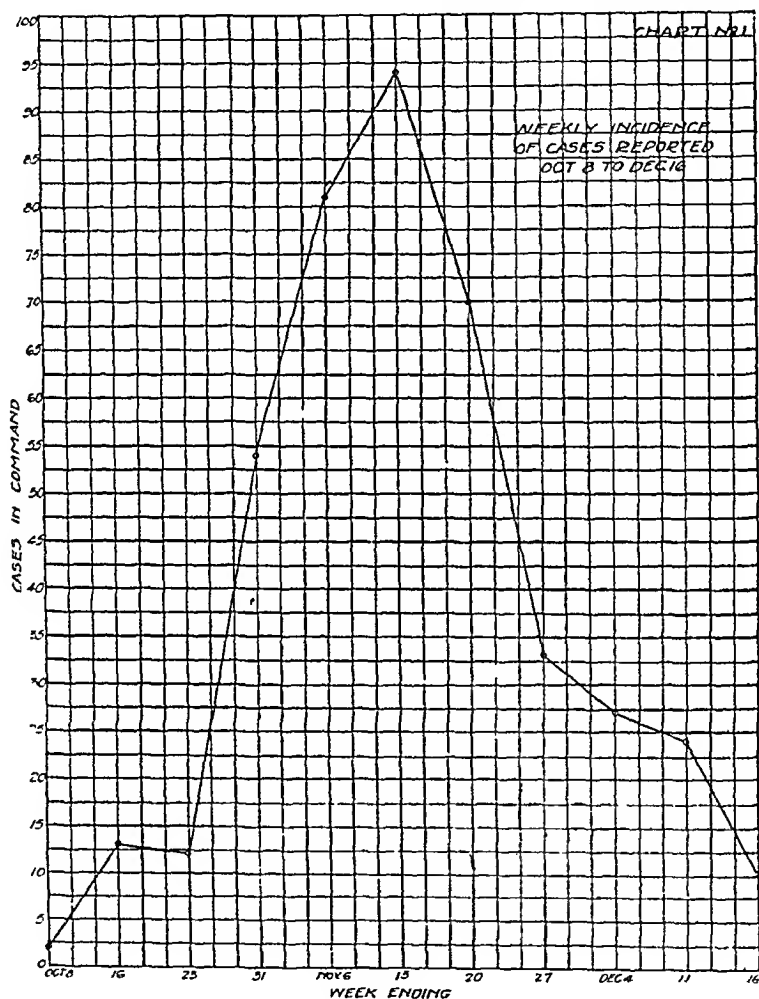
The outbreaks of infectious diarrhea were ascribed to a number of different probabilities, mainly dietary faults. Investigation shows, however, that the disease was infectious in character, spreading from organization to organization, that it was initiated during the summer months, reaching a climax during the late summer and early autumn and finally declining with the beginning of cold weather. A number of investigators believed the outbreaks to be infections with *B dysenteriae*, despite the fact that there was no bacteriologic evidence to support such a view. The endemicity of bacillary dysentery in the A E F may have influenced the formation of such opinion, but in no case of true infectious diarrhea were dysentery bacilli found in a sufficient number of cases to account for the outbreak. A contributory cause of this confusion was the failure to distinguish between infectious diarrhea and bacillary dysentery.

Infectious diarrhea is a mild nonfebrile disease of short duration characterized by symptoms of an irritating enteritis, and a distinction is drawn between that type of outbreak and bacillary dysentery¹.

^{*} From the Department of Preventive Medicine and Hygiene Harvard Medical School.

¹ Though infectious diarrhea is a generic term which includes acute infections with *B dysenteriae*, I would, for purposes of simplicity and convenience, limit the term to the milder diarrheas of unknown etiology and speak of the more severe type caused by the dysentery bacilli as dysentery. The terms are used in that sense in this paper.

The two diseases are clinically distinct. Infectious diarrhea is characterized by a mild diarrhea developing suddenly, and accompanied by cramps in the abdomen and occasionally by vomiting. The cramps are not continuous, but come on spasmodically. There are no marked, general symptoms, such as fever, except that there results a feeling of depression and weakness due to the frequent evacuations. The symptoms are local and purely intestinal. Recovery usually occurs in from three to five days after purgation. A small percentage of cases show more severe reactions, with slight fever, and require hospitaliza-



tion. These exceptionally severe cases are usually discharged recovered in about two weeks. The diarrhea is watery, voluminous and explosive, light to dark brown in color and does not contain blood or mucus. On the other hand, most cases of bacillary dysentery progress rapidly to a much more severe condition, and almost all require hospitalization. The cramps, unlike those in infectious diarrhea, are more continuous and much more severe. There is tenesmus and irritation of the anus. The stools are mucoid to mucosanguineous in character. There is

usually a marked general reaction with fever ranging from 100 to 105 F, and evidences of toxemia. Finally, bacillary dysentery is associated with *B. dysenteriae* which is not found in infectious diarrhea.

Infectious diarrhea is prevalent among the French population during the summer months, and constitutes the largest part of the summer diarrhea of infants. The difference between it and true dysentery is recognized by French physicians, and various local names are used to distinguish it. In St. Nazaire and its vicinity, the disease is known as "la Croisicette" or "la Sablaise," the names being derived from two towns, la Croisic and les Sables d'Olonne, respectively, south and north of St. Nazaire, where it is believed the outbreaks find their endemic focus each summer, and spread to the surrounding country.

Most of the early workers on infectious diarrhea devoted themselves to the identification of organisms in the stools of diarrhea patients. A number attempted to prove an etiologic relationship. Cumston,² in one of the earliest papers on the subject, expressed his belief that *B. coli* can become virulent and so cause infantile diarrhea. Escherich³ isolated a streptococcus that was pathogenic for white mice. Booker,⁴ Baginsky,⁵ Tomkins,⁶ Czerny and Moser,⁷ Lesage,⁸ Hirsh,⁹ Delepine¹⁰ and others, described a variety of organisms representing the bacterial flora in diarrhea cases, but in no case were these investigators able to prove a causal relationship to the disease. Morgan¹¹ isolated a number of organisms, among them some gram-negative bacilli intermediate between the paratyphoid and dysentery organisms which were pathogenic for rats.

Booker¹² concludes that "no single micro-organism is found to be the specific exciter of the summer diarrhea of infants." It is interesting, however, to note his observation in an investigation of ninety-two cases, that "Micrococci are present in all of the cases, in immense numbers in some, being fewer in others. In some cases they fail to grow on culture mediums, but appeared in great numbers in cover-slip preparations from the intestinal contents and in hardened sections of the intestines."

2 Cumston Internat M Mag, February, 1897

3 Escherich Wien klin Wchnschr 1897, No 42

4 Booker Ninth Internat Med Cong, III, p 598, 1887

5 Baginsky Berl klin Wchnschr, 1889, Nos 46, 47 and 49, Arch f Kinderh 12 Pt 2, 1890

6 Tomkins Brit M J 2 180 1889

7 Czerny and Moser Jahrb f Kinderh 38 4 1894

8 Lesage Semaine méd, Oct 16, 1897

9 Hirsh Centralbl f Bakteriöl 22 Nos 14 and 15

10 Delepine, S J Hygiene 3 68, 1903

11 Morgan Brit M J 1 908, 1906

12 Booker Johns Hopkins Hosp Rep 6 1897

Following the identification of the various dysentery bacilli¹³ a number of investigators attempted to determine the relation of these organisms to infant diarrhea. In a series of investigations published in 1904 by Bassett,¹⁴ Gay and Stanton¹⁵ and Duval and Bassett¹⁶ on 417 cases of summer diarrhea, 67 per cent yielded the dysentery bacillus. With very few exceptions they were of the "Harris" type. In the large majority of the cases either blood or mucus was present in the stools, and it is evident that these workers were dealing with dysentery outbreaks. It is interesting, however, that in the cases in which neither blood nor mucous was present, no dysentery bacilli were isolated. Rotch¹⁷ concludes, after an examination of sixty-one cases, only 16 per cent of which showed the presence of dysentery bacilli, that "the infectious diarrheas are probably caused by a variety of organisms. The *B. dysenteriae* has been proved to be one cause, though not yet the sole cause." Collins¹⁸ in "a study of the dejecta of normal children and those suffering from acute and subacute diarrhea, with special reference to *B. dysenteriae*," failed to find the organism present and concluded that it "would lead us to suspect some cause or combination of causes other than this organism as the etiological factor in these conditions."

Other attempts to determine the presence of dysentery bacilli in cases of infant diarrhea were made by Duval and Bassett¹⁹ who found forty-two out of fifty-three cases positive, Wollstein²⁰ who found 39 out of 114 cases positive, Michael²¹ who found twenty-four out of ninety-seven cases positive, Dunn²² who found only ten out of sixty-one cases positive, and Schwartz²³ who failed to find the organism in thirty cases that he investigated. Weaver and Tunnichiff²⁴ found that only 25.4 per cent yielded organisms culturally similar to *B.*

13 Shiga. *Centralbl. f. Bakteriol.* **23** 1898, **24** 1898, *Deutsch. med. Wchnschr.* **43** **44** **45** 1901. Flexner. *Phila. M. J.* **6** 1900, *Bull. Johns Hopkins Hosp.* **11** 1900. Strong and Musgrave. *Rep. Surg.-Gen. U. S. Army Washington*, 1900. Kruse. *Deutsch. med. Wchnschr.* **26** 1900, **27** 1901. Hiss and Russell. *Med. News*, February, 1903.

14 Bassett. *Studies Rockefeller Inst.* **2** 88 1904, Lewis. *ibid.* **2** 82, 1904, Kendall. *ibid.* **2** 76, 1904, Waite. *ibid.* **2** 72, 1904, Duval. *ibid.* **2** 42, 1904, Wollstein. *ibid.* **2** 55, 1904, Cordes. *ibid.* **2** 67, 1904.

15 Gay and Stanton. *Studies Rockefeller Inst.* **2** 61, 1904.

16 Duval and Bassett. *Studies Rockefeller Inst.* **2** 7, 1904.

17 Rotch. *Am. Med.* **2** 737, 1904.

18 Collins. *J. Infect. Dis.* **2** (Nov. 25) 1905.

19 Duval and Bassett. *Am. Med.* **4** 417 (Sept. 13) 1902.

20 Wollstein. *J. M. Research* **10** 11, 1903.

21 Michael. *J. Infect. Dis.* **2** (Jan. 12) 1905.

22 Dunn. *Am. Med.* **7** 737, 1904.

23 Schwartz. *Proc. New York Path. Soc.* **3** 172 1903.

24 Weaver and Tunnichiff. *J. Infect. Dis.* **2** (Jan. 12) 1905.

dysenteriae out of a series of 102 cases. They conclude that "we are unacquainted with a specific cause for all cases of summer diarrhea in infants."

English workers²⁵ appear to have been more precise in distinguishing between epidemic diarrhea and bacillary dysentery, and concerned themselves more particularly with the question of the source of these outbreaks and the transmissibility of the disease. The general consensus of opinion of these English investigators points to the "fly carrier" as the most probable line of communication.

Taylor and Knapp, in a report to the base surgeon, Base Section No. 1, on the gastro-intestinal disorders occurring in the vicinity of St. Nazaire indicate the presence of two distinct types. They regard all cases which show blood and mucus as probably being due to *B. dysenteriae*, and are of the opinion that the milder type is of an infectious nature, the etiology being unknown.

FIELD INVESTIGATION

In a field investigation made Dec. 15 and 16, 1918, I found that a considerable number of cases of diarrhea had occurred in and around the camp at St. Anne on the outskirts of Nantes. A number of these

TABLE 1—NUMBER OF CASES OF DIARRHEA REPORTED, ST. ANNE BARRACKS, NANTES (SEPTEMBER)

Organization	September, 1918										
	4	5	6	7	8	9	10	11	12	14	16
824th Co. 826th Labor Btn		3			1	1	1			2	1
Co. A, 306th Labor Btn	1						1		1		
Det., Co. C, 513th Engrs	1		2	1	1			1		1	
Total	2	3	2	1	2	1	2	1	1	3	1

had occurred before and during the month of August of which there were no available records. The "sick books" showed that nineteen cases occurred during the early part of September, three organizations being involved. These were the first recorded cases and were reported as shown in Table 1.

The occurrence of these cases indicated at that time nothing of epidemiologic significance. They probably formed the initial outbreak from which the subsequent epidemic took its focus. No record was kept of any cases that may have occurred during the latter part of September. Two new cases occurred during the first week of October, and from then on there was a steady increase until the week ending

²⁵ Notter. Proc. Roy. Soc. Med., Lond., Epid. Sect., Pt. 2, p. 263, 1910, Niven. *ibid.* 3, Pt. 2 (April 7) 1910, Sandilands. *ibid.* Feb. 25, 1910, Parsons, *ibid.*, Pt. 2, p. 269 (June) 1910, Peters. J. Hygiene 10, 1910, Newsholme. Practitioner, August, 1912.

November 14 when ninety-four new cases were reported. A gradual decline followed. During the week ending December 11, twenty-four new cases occurred and from December 12 to 16, ten cases were reported. The cases and their corresponding organizations are given in Table 2. A graphic representation of this outbreak is given in the accompanying chart.

The primary sporadic outbreak and the subsequent epidemic following about two weeks later indicated some infectious agent which gradually spread through the various organizations, as shown in Table 2. During the first week one organization only was affected, during the second week it spread to three more and during the third, fourth and fifth weeks to two, three and two more respectively until ultimately the whole command with the exception of a Chinese battalion of 182 men had developed cases.

TABLE 2—CASES OF DIARRHEA REPORTED BETWEEN OCTOBER 1 AND DECEMBER 16*

Organization	Total Strength	Week Ending										Dec 12 to Dec 16
		October				November				December		
		8	16	23	31	6	13	20	27	4	11	
806th Co 824th Lab Btn	226	2	7	6	13	23	25	22	6	4	3	0
Co D, 308th Lab Btn	246	0	2	1	6	10	12	5	5	1	6	0
Co A, 306th Lab Btn	138	0	3	1	17	22	9	2	4	4	3	2
Casual Co, No 1	26	0	1	2	7	7	8	6	3	4	2	6
Co C, 518th Engrs	57	0	0	1	2	2	5	6	2	0	7	0
Co B, 516th Engrs	40	0	0	1	1†							
Co B, 315th Lab Btn	45	0	0	0	3	5	3	3	1	1	0	0
Q M C Detachment	150	0	0	0	1	2	2	7‡				
336th M G Bat Cos O & D	320				4§							
Co C 701st Stev Btn	169	0	0	0	0	11	11	8	4	1	0	0
Co A, 315th Lab Btn	127	0	0	0	0	1	19	11	8	12	3	2
Totals	1,544	2	13	12	54	83	94	70	33	27	24	10

* Total cases, 422, morbidity rate, 27.3 per cent

† Left camp after week of October 31

‡ Left camp after week of November 20

§ In camp only two weeks

A sanitary survey was made, the barracks, latrines and kitchens being examined. Particular attention was given to the mess, especially as regards the difference between the food served and methods used by the affected organizations and the nonaffected Chinese battalion, the assumption being that there might be more than a difference in racial resistance to explain it. It was found that practically nothing was served in the Chinese mess which had not previously been boiled. The ration consisted of a regular diet of meat soup, rice and tea. Raw water was never used, tea being substituted. The water was the same as that supplied to the city of Nantes in which no considerable outbreak of diarrhea had been observed.

In all the kitchens a large number of flies were found for the season of the year, and inquiry determined that they had been a considerable pest until about four weeks previous to the investigation. This evidence and the fact that the initial outbreak occurred during the height of the fly season and that the epidemic began to wane immediately after the fly season ended, and kept continually dropping off as they disappeared, and the particular manner in which the disease spread from organization to organization lends countenance to the possibility that they were a channel of infection. Though the closets in which bread and meat were kept were sufficiently well screened, no attempt was made to screen the kitchens themselves and exposed food was in that manner unprotected.

LABORATORY INVESTIGATION

Specimens of blood, urine and feces were obtained from eight cases. The course of the disease was relatively mild, only fifteen patients out of a total of 422 requiring hospitalization. Of these, six had been discharged prior to this investigation. One had developed pneumonia and was unavailable for experimental study.

TABLE 3—CASES AND ISOLATIONS

Case	Name	Ward	Source of Isolations		
			Feces	Urine	Blood
1	Williams	A10	Negative	15, 16, 17	Negative
2	Clark	A 9	Negative	20, 21	Negative
3	Gathright	A 9	25, 26	27, 28	30
4	Hodgkins	A 9	Negative	Negative	Negative
5	Bowden	414	3, 4	11, 12, 13	Negative
6	Ashford	416	1, 2	9, 10	Negative
7	Langford	A10	5, 6, 7, 8	Negative	Negative
8	Williams	414	Negative	18, 19	Negative

The numbers designate strains

Isolation of the Organisms—The specimens were plated on a carefully balanced neutral Endo medium containing the minimum amount of fuchsin and sodium bisulphite commensurate with a good color reaction. A gram-positive micrococcus was isolated in four cases from the feces, in six cases from the urine and in one case from the blood. Seven cases yielded this coccus from one or more sources, in several instances in almost pure culture. One convalescent case was negative (Table 3). The colonies appear as small colorless droplets after overnight incubation, that later grow pink, due to slow fermentation of the lactose.

Morphology and Growth—The organism is a small gram-positive micrococcus varying considerably in the size of the individuals and in the degree of retention of the gram stain. The larger forms show a tendency to take the counter stain. They are irregularly round and

flattened at the adjacent surfaces, occurring in diploforms or in aggregates of diploforms. The colonies are small, colorless, to slightly bluish and transparent becoming later grayish and umbilicated and present a "dry surface" appearance as they get older. The coccus develops readily on neutral infusion agar, but never produces more than a delicate confluent growth.

TABLE 4—FERMENTATION REACTIONS

Fermentation Mediums Containing	Strains							
	No 19	No 11	No 16	No 26	No 15	No 30	No 28	No 4
Dextrose	Acid	Acid	Acid	Acid	Acid	Acid	Acid	Acid
Mannite	Acid	Acid	Acid	Acid	Acid	Acid	Acid	Acid
Maltose	Acid	Acid	Acid	Acid	Acid	Acid	Acid	Acid
Lactose	Acid	Acid	Acid	Acid	Acid	Acid	Acid	Acid
Sucrose	Acid	Acid	Acid	Acid	Acid	Acid	Acid	Acid
Xylose	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn
Rhamnose	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Inosite	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Arabinose	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn
Inulin	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Dextrin	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn	Rdn
Raffinose	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Saline	Acid	Acid	Acid	Acid	Acid	Acid	Acid	Acid

Rdn = reduction

Fermentation Reactions—The strains were tested on dextrose, mannite, maltose, lactose and sucrose broth. They fermented all with acid, but no gas formation. No other fermentable substances were available at that time, but subsequent tests carried out on a larger variety of mediums with eight strains successfully transported to the United States showed that the strains were identical in their biological activity. The results are given in Table 4.

TABLE 5—AGGLUTINATION OF PATIENT'S SERUM WITH
HOMOLOGOUS STRAINS CASE 1

Source of Strain	Strain No	Serum Dilutions					Human Serum Control 1 10	Salt Solution Control
		1 10	1 20	1 40	1 80	1 160		
Urine	15	++	++	++	+	+	—	—
		++	++	++	++	++	—	—
Urine	17	++	++	++	++	++	—	—
		++	++	++	++	++	—	—

± indicates slight agglutination, + indicates agglutination without sedimentation, and ++ indicates agglutination with sedimentation.
Upper readings taken after 8 hours incubation
Lower readings taken after 18 hours incubation

Agglutination reactions Serums obtained from four cases during the period from the tenth to the fourteenth day of the disease were tested against the organisms isolated from these cases. The agglutinations were carried out at 37.5 C (water bath) and readings were taken after eight and eighteen hours. The results are given in Tables 5 to 8 inclusive.

TABLE 6—AGGLUTINATION OF PATIENT'S SERUM WITH
HOMOLOGOUS STRAINS CASE 2

Source of Strain	Strain No	Serum Dilutions					Human Serum Control 1 10	Salt Solution Control
		1 10	1 20	1 40	1 80	1 160		
Urine	20	+	++	++	++	+	—	—
		++	++	++	++	++	—	—
Urine	21	±	+	+	++	+	—	—
		++	++	++	++	++	—	—

Upper readings taken after 8 hours incubation
Lower readings taken after 18 hours incubation

TABLE 7—AGGLUTINATION OF PATIENT'S SERUM WITH
HOMOLOGOUS STRAINS CASE 5

Source of Strain	Strain No	Serum Dilutions					Human Serum Control 1 10	Salt Solution Control
		1 10	1 20	1 40	1 80	1 160		
Feces	3	±	+	+	++	+	—	—
		++	++	++	++	++	—	—
Feces	4	++	++	++	++	+	—	—
		++	++	++	++	+	—	—
Urine	11	±	+	++	++	++	—	—
		++	++	++	++	++	—	—
Urine	12	±	+	+	++	+	—	—
		—	—	—	—	—	—	—
Urine	13	±	+	+	±	±	—	—
		±	++	++	+	+	±	±

Upper readings taken after 8 hours incubation
Lower readings taken after 18 hours incubation

TABLE 8—AGGLUTINATION OF PATIENT'S SERUM WITH
HOMOLOGOUS STRAINS CASE 6

Source of Strain	Strain No	Serum Dilutions					Human Serum Control 1 10	Salt Solution Control
		1 10	1 20	1 40	1 80	1 160		
Feces	1	—	±	±	±	+	+	—
		+	+	+	+	+	±	±
Feces	2	±	±	±	±	±	—	—
		+	+	±	±	±	—	—
Urine	9	±	±	±	±	±	—	—
		+	++	+	+	+	—	—
Urine	10	±	±	±	±	±	—	—
		±	±	±	+	+	—	—

Upper readings taken after 8 hours incubation
Lower readings taken after 18 hours incubation

It is evident from the series of agglutination reactions given in Tables 5 to 8 that the serum of persons recovering from the disease

contains specific agglutinins against the strains isolated from the same cases

Cross agglutinations were next carried out, using the two most potent human serums (Cases 1 and 2) against all the heterologous strains isolated from other cases. The results are given in Tables 9 and 10

TABLE 9—CROSS AGGLUTINATIONS SERUM FROM CASE 1

Strain No	Source	Serum Dilutions				Salt Solution Control
		1 20	1 40	1 80	1 160	
1	Feces	+	+	+	+	±
2	Feces	—	±	±	±	—
9	Urine	++	±	—	—	—
10	Urine	+	±	—	—	—
3	Feces	±	±	±	—	—
4	Feces	+	+	+	+	—
11	Urine	+	+	+	—	—
12	Urine	+	+	+	—	—
13	Urine	+	+	+	+	+
5	Feces	+	+	±	±	—
6	Feces	++	±	—	—	—
7	Feces	++	+	—	—	—
8	Feces	++	+	—	—	—
18	Urine	+	+	+	+	—
19	Urine	—	±	+	±	—
20	Urine	—	±	+	±	—
21	Urine	—	+	+	—	—
25	Feces	+	+	+	+	—
26	Feces	—	+	±	—	—
27	Urine	—	+	+	+	—
28	Urine	—	—	—	—	—
30	Blood	—	—	—	—	—

TABLE 10—CROSS AGGLUTINATIONS SERUM FROM CASE 2

Strain No	Source	Serum Dilutions			Salt Solution Control
		1 40	1 80	1 160	
1	Feces	++	+	+	±
2	Feces	++	++	++	—
9	Urine	++	++	++	—
10	Urine	++	++	++	—
3	Feces	++	++	++	—
4	Feces	++	++	++	—
11	Urine	+	+	++	—
12	Urine	++	++	++	—
13	Urine	+	+	+	±
5	Feces	++	++	++	—
6	Feces	++	+	+	—
7	Feces	++	+	+	—
8	Feces	++	+	—	—
15	Urine	—	—	—	—
16	Urine	—	+	+	—
17	Urine	+	—	—	—
18	Urine	+	+	+	—
19	Urine	±	±	±	—
25	Feces	++	++	++	—
26	Feces	++	+	—	—
27	Urine	±	+	—	—
28	Urine	—	—	—	—
30	Blood	—	—	—	—

Of the twenty-five strains tested against the two heterologous serums, all but three (Strains 15, 28 and 30) were agglutinated. These three strains were among the eight surviving strains transported to this country and were subsequently tested after a series of transplants

against a highly potent rabbit serum. All three were agglutinated (Table 11)

Animal Experiments Attempts to reproduce the disease in rabbits failed. Young and old rabbits were fed with mixed cultures, the results were uniformly negative.

Macacus rhesus monkeys were next used with the following results

MONKEY No 1—A *Macacus rhesus* monkey was fed 10 cc of mixed broth cultures of eight strains on three successive days. Twelve days after the last feeding the animal showed signs of illness with very profuse diarrhea on the thirteenth day. He died on the fourteenth day.

Feces collected on the thirteenth day were plated. A great many characteristic colonies developed which showed the typical micrococcus in smear preparation. Six fishings proved to be identical by fermentation and agglutination reactions with the organism previously described.

At necropsy, the lungs, heart, spleen, kidneys and gallbladder were found normal. The mesentery was considerably injected, and showed a hyperemia indicating an acute condition. The mesenteric lymph nodes were found con-

TABLE 11—AGGLUTINATION REACTIONS, RABBIT SERUM (STRAIN 5 CASE 7)

Case	Strain No	Dilutions			Salt Solution Control
		1:100	1:1,000	1:10,000	
1	15	++	±	—	—
1	16	++	±	—	—
3	26	++	+	—	—
3	28	++	—	—	—
3	30	++	—	—	—
7	4	++	++	++	—
7	11	++	+	—	—
8	19	++	—	—	—

siderably reddened. There was slight hyperemia of the small intestine. The organism was present in direct smear preparation and section in the mesenteric lymph nodes and spleen. It was isolated in pure culture from mesenteric lymph nodes and heart's blood.

MONKEY No 2—A second monkey was subsequently fed with the living bacteria filtered free from the broth in which they had been grown and incorporated as part of a banana paste. The animal was fed on three successive days with the bacteria filtered from 10 cc of culture. On the fifth day he developed a diarrhea but rapidly recovered.

MONKEY No 3—A third monkey was fed on three successive days with 10 cc of mixed broth cultures by means of a stomach tube. He died eight days after the first feeding. At necropsy a pathologic condition was found at the juncture of the large and small intestine which resembled an acute colitis and a small patch was found in the wall of the small intestine. There was marked injection along the colon. Micrococci were found in the mesenteric lymph nodes and spleen.

MONKEY No 4—One monkey was fed on three successive days with 10 cc quantities of the sterile filtrate from twenty-four hour broth cultures. The feeding was carried out by means of a stomach tube. The animal remained normal and showed no symptoms.

MONKEY No 5—This animal was similarly fed, but bacteria killed by heating at 60 C for twenty minutes were used. The monkey remained normal and showed no symptoms.

MONKEY No 6—This monkey was actively immunized with a polyvalent bacterial vaccine made from the eight cultures and killed at 60 C for twenty minutes. Three doses of 1 cc each were given subcutaneously at intervals of one week. Two weeks later the animal was fed 10 cc of mixed cultures of the living bacteria on three successive days by means of a stomach tube. No symptoms developed.

MONKEY No 7—It was attempted to passively immunize this monkey by means of rabbit sera produced against the eight strains. Four cc of the pooled rabbit serum was given intraperitoneally. The animal was fed with 10 cc of the mixed broth cultures by means of a stomach tube on three successive days. Death occurred fifteen days after the first feeding. The necropsy showed the lung, liver and kidneys to be normal. There was definite injection along the colon and the omentum was hemorrhagic. The mesenteric lymph nodes were slightly reddened. Micrococci were present in the spleen and mesenteric lymph nodes by direct smear and in section. The organisms were isolated from both sources and were proved identical by agglutination.

SUMMARY AND CONCLUSIONS

1 Infectious diarrhea, as defined, and dysentery are two distinct diseases, differentiated clinically and bacteriologically. The main clinical differences are that infectious diarrhea is a mild disease of short duration with local manifestations and no general symptoms. The symptoms are chiefly intestinal. Blood or mucous is not present in the stools. Bacillary dysentery is usually very much more severe and of longer duration, there is a marked general reaction with fever and evidence of toxemia. The stools usually contain blood or mucous. For our present purpose we have defined dysentery as that disease caused by *B. dysenteriae*, and infectious diarrhea as that variety of outbreaks which are more mild and are at present of unknown etiology.

2 Infectious diarrhea is probably transmitted in much the same manner that other enteric diseases are spread, one of the agencies being flies.

3 Of eight cases in which urine, feces and blood were examined, seven yielded a gram-positive micrococcus from one or more sources, which were biologically identical in their reactions on fermentation mediums. The organisms were agglutinated by the serum of patients from which they were obtained, and also by the serum of other patients. Three strains that did not agglutinate when first isolated were subsequently agglutinated by rabbit serum produced with a heterologous strain.

4 Rabbits are resistant to infection by this organism. Old and young rabbits were used, but the results were uniformly negative.

5 *Macacus rhesus* monkeys were fed with mixed strains. One monkey died on the fourteenth day after a profuse diarrhea on the thirteenth day from which the organism was isolated. It was subsequently isolated at necropsy from the mesenteric lymph nodes and the spleen. A second monkey fed with cultures, filtered free from the

broth in which they had been grown, developed a diarrhea on the fifth day and recovered. A third monkey was fed with mixed broth cultures by means of a stomach tube. He died on the eighth day. A pathologic condition was found at the juncture of the large and small intestine and the organism was found in the mesenteric lymph nodes and spleen.

Two monkeys, one fed with the sterile filtrate from broth cultures and the other with cultures killed by heating at 60 C. for twenty minutes developed no symptoms and remained alive.

One monkey actively immunized by a polyvalent vaccine made from these cultures was subsequently resistant to infection.

One monkey that we attempted to immunize passively by injection with specific rabbit serum died fifteen days after feeding, and the organism was isolated at necropsy from the spleen and mesenteric lymph nodes.

These experiments indicate that the organism is pathogenic for *Macacus rhesus* monkeys, produces no exotoxin or endotoxin and can be used to produce active immunity against subsequent infection.

6 The sum of evidence presented in this paper suggests the etiologic relationship of the organism described to infectious diarrhea in the outbreak studied.

I wish to acknowledge my indebtedness to Mr. Robert V. B. Emmons, Private U. S. Medical Corps, who aided me materially in the early part of this work in France, to Prof. M. J. Rosenau whose interest and aid enabled me to carry it on to a conclusion in this country, and to Prof. J. Bronfenbrenner for his valuable constructive criticism. Miss Lila Spence has given me her technical assistance and I thank her for her kind and efficient help.

THE INTRAVENOUS USE OF FOREIGN PROTEIN IN THE TREATMENT OF CHRONIC CASES OF ARTHRITIS

WITH SPECIAL REFERENCE TO THE USE OF SECONDARY PROTECSE^{*}

R GARFIELD SNYDER, M.D., AND MAXIMILIAN A. RAMIREZ, M.D.
NEW YORK

In a paper already published by one of us,¹ the value of intravenous injections of typhoid vaccine in acute arthritis was discussed. In the present study, we have confined ourselves to the determination of the value of foreign protein in the treatment of the more obstinate chronic cases of arthritis. The observations contained in this paper cover a period of four years, and during that time we have endeavored to find answers to a number of important questions relative to this subject.

We have attempted to determine whether it is possible to actually and permanently cure cases of chronic arthritis by this method of treatment, or whether, in the absence of a cure, it is possible to improve the patient's condition without at the same time doing him harm. In using the word "cure" we wish to convey the meaning of decided improvement of motion, and cessation of arthritic pain.

During the past four years, we have had charge of seventy cases of chronic arthritis. Of these, sixty were hospital cases, and ten were private cases. We have had three presumable cures in the hospital cases, and three in private practice. Of the hospital cases, one patient has been free from arthritic symptoms for three years and two patients have been symptomless for two years, of the private cases, two patients have been free from arthritic symptoms for two years and one patient for fifteen months. These cases have not been observed for a sufficiently long time to make us certain that the cure has been permanent, but their condition is, up to date, most satisfactory. All of the six patients mentioned as cured are working at present, and earning their living, whereas, previously, they had been helpless invalids. Two of the patients still have a slight amount of deformity due to the presence of firm bands of adhesions and bony changes in the joints. One could hardly expect foreign protein to alter firm fibrous adhesions or bony changes in and around the involved joints. Undoubtedly, the violent shaking of all the joints during the chill following the administration of the protein causes the breaking up of numerous fine adhesions in the joints and their associated tendinous sheaths. The degree of

^{*}From the First Medical Division, City Hospital.

1 Snyder, R. G. A Clinical Report of Nonspecific Protein Therapy in the Treatment of Arthritis, *Arch. Int. Med.* 22:224 (Aug.) 1918.

improvement in these cases is, in part, due to the breaking up of these fine adhesions and release of muscle spasm following relief from pain.

The average percentage of cures in this series of chronic arthritis was about 85. In reviewing them, we have been impressed by the fact that all our "cures" occurred in patients who had suffered from arthritic symptoms for less than two years.

It would appear that the improvement in these cases was directly due to the foreign protein therapy, as they all had previously been given the routine treatment for chronic arthritis without any definite improvement in their symptoms. The routine treatment consisted of application of various liniments to the joints, rest in bed, the removal of all apparent foci of infection, special diets, and baking.

Of the patients who were only improved, the majority had suffered from arthritis for several years. In many instances, their joints showed well marked deformities and bony changes. The degree of benefit to be obtained varies with the individual case, and is nearly always greatest in the joints of the upper extremities. Here 50 per cent showed considerable improvement in motion and decrease in pain. In the joints of the lower extremities, the results are not so good, only 25 per cent of these cases showed slight improvement in motion and pain. However, as these patients are, as a rule, helpless invalids, they are usually extremely grateful for any improvement, no matter how slight.

The intravenous foreign protein treatment is contraindicated in cases of chronic arthritis complicated by any form of tuberculosis, extreme emaciation from any cause, cardiac decompensation and excessive hypertension.

The size of the dose is always an important question to be decided. Large doses which produce a marked febrile reaction and a very severe chill cannot be given without a corresponding risk to the patient. Several deaths are known to have occurred as a result of too large doses. In order to minimize this danger, we have tried to determine whether the beneficial results obtained were due to the production of the chill or to the size of the dose of foreign protein. After considerable experimentation, it became apparent that the chill is the important guiding factor, consequently there is no advantage in increasing the dose of protein with each injection. If the initial dose is large enough to produce a chill, it is not necessary to increase the dose as long as it continues to give a satisfactory reaction. In our series, the dose used was ten million typhoid bacilli, or from $\frac{1}{2}$ to 1 gram of secondary proteose ².

2 The secondary proteose employed in this series of cases was prepared from milk, and was obtained through the courtesy of the Arlington Chemical Company.

Most of the patients with chronic cases are poorly nourished, and the injection should, therefore, be given only once a week to prevent rapid loss of weight. Since each injection should be preceded by thorough catharsis and is usually followed by nausea for several hours, each treatment means almost twenty-four hours of complete abstinence from food. By observing the precautions in regard to the contraindications, the size of the dose and the interval between injections, none of our patients showed any ill effect. Some of the patients had as many as from fifty to seventy-five injections of foreign protein.

The foreign protein most commonly used in this country has been typhoid vaccine, on account of its accessibility, the dose is estimated by the number of bacilli injected. The use of any vaccine as foreign protein for intravenous use has certain disadvantages—for instance

- 1 The dose is uncertain, inasmuch as bacterial counts in vaccines are only approximate,³ and the protein content of different types of bacteria is variable.⁴

- 2 The introduction into the circulation of endotoxins in large quantities is to be avoided, if possible. Since all vaccines contain both protein and endotoxins, the elimination of endotoxins as a dangerous factor can only be accomplished by the use of secondary proteose.

- 3 There is always a slight danger of introducing live organisms into the circulation even when the vaccines are prepared in the most reliable laboratories.

- 4 All bacteria contain potentially both primary and secondary proteoses and it has been shown that the primary are more toxic than the secondary proteoses.

Osborne and Wells⁵ have shown that it is impossible to produce anaphylactic shock by the use of secondary proteose. Since fatalities have been known to occur, following the intravenous administration of large doses of typhoid vaccine, we believe that the presence of primary proteose in the vaccine is in all probability a source of constant danger. The primary proteose may be present in the vaccine used, as a result of autolysis, or may be liberated in the circulation after inoculation, as a result of the natural splitting of the bacterial protein molecule.

3 McCoy, G. H. Hygienic Laboratory Bulletin No. 110

4 Vaughn, Victor C. Poisonous Proteins, St. Louis, C. V. Mosby Company

5 Osborne and Wells. The Anaphylactic Reaction with So-Called Proteoses of Various Seeds. The Biological Reactions of the Vegetable Proteins, J. Infect. Dis. **17** 259 (July) 1915

SUMMARY

The majority of the patients in this series have at one time or another during the past three years had both typhoid vaccine and secondary proteose administered intravenously. They have experienced less nausea, headache and weakness following the use of secondary proteose than after the injection of typhoid vaccine. Over a long series of injections they seem to lose less weight with the use of secondary proteose than with the typhoid vaccine. On the whole, the proteose appears to be less toxic, and it is of significance that patients who have been treated with both typhoid and proteose strongly prefer the proteose for subsequent injections.

In relieving the pain and in improving the motion in the joints, equally good results seemed to follow the use of proteose and typhoid vaccine.

Cases of chronic arthritis which have not been relieved by the routine treatment consisting of sodium salicylate, acetylsalicylic acid, colchicin, cincophen, hot packs, baking or massage are, as a rule, materially benefited by the intravenous administration of small doses of foreign protein. The degree of benefit to be obtained varies with the individual case, and is nearly always greatest in the joints of the upper extremities.

The writers are indebted to Drs. Bastedo, McCabe, Qumby, Shelby, Brooks, Whitman and Bradbury for the use of cases in compiling this article.

RENAL GLYCOSURIA ~

PHIL L MARSH, MD

ANN ARBOR, MICH

While the majority of clinicians have admitted the existence of renal glycosuria, the number of entirely acceptable cases that have been reported is small. Allen¹ accepts only two, those of Bonninger² and Tachau,³ and Foster⁴ found no others above suspicion. Recently, several cases have been reported, some of which present the characteristics of mild diabetes mellitus. With the introduction of simple, accurate methods for determining the sugar content of the blood, it has been possible to establish the diagnosis on a firm basis.

The subject has been discussed in detail by several modern authors, notably Goto,⁵ Bailey⁶ and Lewis and Mosenthal.⁷ It is important to remember that extreme caution should be used in making the diagnosis of renal glycosuria, rather than that of diabetes mellitus, the disastrous injustice of assuring a diabetic that he has the innocuous disease that the former seems to be, and of failing to regulate his diet, is apparent. The diagnosis depends on (1) glycosuria without hyperglycemia, (2) little, if any, relationship between the amount of sugar excreted and the amount of carbohydrate ingested, and (3) the absence of diabetic symptoms. To these Strouse⁸ adds (4) that the patient must not subsequently develop diabetes mellitus, or show a disturbance of carbohydrate metabolism similar to that found in diabetes mellitus.

It is obvious that the abnormal substance in the urine must be shown to be glucose. It is also important, as pointed out by Bailey,⁶ that the urine and blood be collected simultaneously, to make it certain that the glycosuria actually occurs without hyperglycemia. In the case reported by Murlin and Niles,⁹ for example, the urinary specimens were collected throughout the day and the blood sugar

* From the Department of Internal Medicine, Medical School, University of Michigan

1 Allen, F. M. Diabetes and Glycosuria, Boston, 1912

2 Bonninger, M. Beitrag zur Frage des Nierendiabetes. Deutsch. med. Wchnschr. **34** 780, 1908

3 Tachau, H. Beitrag zum Studium des Nierendiabetes. Deutsch. Arch. f. klin. Med. **104** 448, 1911

4 Foster, Diabetes Mellitus, Philadelphia, 1915, p. 156

5 Goto, K. Alimentary Renal Glycosuria, Arch. Int. Med. **22** 96 (July) 1918

6 Bailey, C. V. Renal Diabetes, Am. J. M. Sc. **157** 221, 1919

7 Lewis, D. S., and Mosenthal, H. O. Renal Diabetes, Bull. Johns Hopkins Hosp. **27** 133, 1916

8 Strouse, S. Renal Glycosuria, Arch. Int. Med. **26** 768 (Dec.) 1920

9 Murlin, I. R., and Niles, W. L. Renal Glycosuria, Am. J. M. Sc. **153** 79, 1917

determinations were made five hours after breakfast, at the time when many mild diabetics might well have a normal glycemia. The relationship between the intensity of the glycosuria and the diet varies somewhat in the cases recorded, in some cases, the two were entirely independent, in others, the changes in the former were in the same direction as changes in the latter, but not proportional to them. The absence of diabetic symptoms would seem to be the most important factor in the diagnosis, and yet some observers have accepted as examples of renal glycosuria cases in which there were definite symptoms of the graver disorder. The patient of Murlin and Niles⁹ had a history of funiculosis, polyuria, thirst and loss of weight. Galambos' patient¹⁰ had polyuria and polydipsia. Only a few cases were under observation for a sufficient length of time to warrant any opinion concerning the possible evolution into diabetes mellitus, chief among these are those of Boenninger,² Graham,¹¹ Paullin,¹² Goto,⁵ Garrod¹³ and Strouse.⁸

Because of the paucity of our knowledge of this remarkable anomaly, it seems desirable to add our observations to those already found in the literature. This case is of especial interest because of the enormous quantities of sugar excreted.

REPORT OF CASE

The patient (No 20-658), a married negress, 24 years of age, entered the hospital Oct 14, 1920, with several indefinite complaints, chiefly pain in her right chest.

Family History—Her father died at the age of 41 of "kidney trouble" and her mother at the age of 39 of "heart trouble with dropsy." Four brothers and three sisters are in good health, there is no history of familial disease, and she knows of no one in her family who had diabetes.

Previous History—She was married at the age of 22, and her husband is in good health. She has never been pregnant. She had measles, mumps and chickenpox in childhood, with good recoveries from all three. For two months at the age of 12, she had attacks of sharp pain in both chests. Throughout her life she had frequent attacks of tonsillitis, with quinsy five years ago, her tonsils were removed three years ago. Her teeth have caused her a great deal of trouble. Headaches have been common and severe.

She admits having had sexual intercourse since the age of 15, but denies gonorrhea and syphilis, and gives no history suggestive of either, except an operation for "appendicitis" at the age of 16, since when she has not menstruated. Her appetite has always been good, but never unusual. For the past five months she has had some nocturia but no polyuria.

January, 1920, she had influenza, followed by pneumonia. At this time she was in bed for about three months, with a severe cough, a moderate irregular fever and considerable sputum, which was frequently dark colored but never

10 Galambos, A. Ueber den renalen Diabetes, Deutsch med Wchnschr **51** 1792, 1914.

11 Graham, G. Variations in the "Leak Point" in Diabetes. I. A. Low Level, J. Physiol **49** 46 (Proc.) 1915.

12 Paullin, J. E. Renal Glycosuria, J. A. M. A **75** 214 (July 24) 1920.

13 Garrod, A. E. Brit. M. J. **2** 850, 1913.

red Associated with this illness, and persisting to the present time, she has had recurring attacks of sharp, shooting pain in her lower right chest, worse on inspiration, and entirely absent during the intervals She believes that during the month after she was allowed out of bed she had a slight afternoon fever, and she has had drenching night sweats two or three times a week There is no cough at present During the attack of pneumonia, her left ankle became red, swollen and painful, the redness and swelling disappeared, but she still complains of soreness There have also been occasional attacks of sharp, shooting pains in the legs and left arm, coming chiefly at night, and lasting only a few minutes For the last five or six months she has been troubled by palpitation of the heart and shortness of breath on moderate exertion Before she had pneumonia, she weighed 139 pounds, after getting out of bed, she weighed 112, her present weight is 122

Physical Examination—On examination, the patient was found to be a slender, fairly well nourished young negress, lying comfortably on the examination table, in no apparent pain or discomfort She was mentally alert, and answered questions promptly and intelligently Physical examination revealed no important evidence of pathology Her teeth were in very poor condition, and many were carious Careful examinations of her chest by several members of the staff were entirely negative There were a few firm, painless inguinal glands felt on each side Her fundus was reported from the department of ophthalmology as practically normal

Blood—Hemoglobin, 90 per cent on the Talquist scale, red blood cells, 5,000,000, leukocytes, 8,400, of which 61 per cent were polymorphonuclears 13 per cent small lymphocytes, 20 per cent large lymphocytes and 6 per cent large mononuclear cells Blood pressure was 115 systolic, and 65 diastolic

Urine—Urine was pale yellow, slightly cloudy, and acid, specific gravity, 1.050, no albumin, a few epithelial cells, and oxalate crystals, a drop of it reduced Fehling's solution rapidly and completely, the ferric chlorid reaction was negative

Two determinations of blood sugar on the day of admission were 0.090 and 0.091, respectively

Roentgenologic examination of chest was entirely negative

Clinical Course—During her stay in the hospital she was afebrile, except during an attack of acute intestinal obstruction Her pulse rate varied between 80 and 100, and her respiratory rate between 20 and 25 She was treated at first as a diabetic, and it was not until several determinations showed that her blood sugar was persistently normal, and after her heavy glycosuria had persisted through several days of a low carbohydrate diet, that a serious doubt as to the diagnosis of diabetes mellitus was entertained The study of the sugar content of her blood and urine after the ingestion of glucose was convincing evidence that it was not a case of typical diabetes

The substance in the urine was found not only to reduce Fehling's solution and Benedict's qualitative reagent, and to give a satisfactory endpoint with Benedict's quantitative solution, but it also gave the other important reactions of glucose It was fermented rapidly by yeast, with the production of gas, there was no gas production by the yeast in the control urines Typical phenolhydrazine crystals were thrown down by the Kowarski method Polarized light was rotated to the right, and glucose estimations by the polariscope agreed closely with those by Benedict's reagent

Urinary glucose was determined throughout the study by the Benedict method and the blood sugar by Benedict's modification of the Lewis-Benedict method

Comment—The relation of her urinary sugar excretion to her diet is shown in Table 1 During the periods of observation, she was on six dietary regimes From October 5 to October 20, inclusive, she

TABLE 1—RELATION OF DIET TO URINARY SUGAR

Date	Diet				Blood Sugar, per Cent	Urine			Remarks
	Protein, Gm	Fat, Gm	Carbohydrate, Gm	Calories		Amt, Cc	Sp Gr	Glucose, Gm	
10/15	20	84	13	890	0.090		1.050	+	Single specimen Low protein Low carbohydrate diet
10/16	19	106	16	1,043			1.039	+	
10/17	20	74	15	897		1,120	1.039	43.2	
10/18	20	83	15	884	0.090	950	1.042	36.0	
10/19	22	92	12	967		1,030	1.043	41.1	
10/20	15	73	15	782	0.100	960	1.038	44.9	
10/21	100	137	331	2,960		1,720	1.038	57.7	Unrestricted diet
10/22	119	85	356	3,069		1,085	1.044	67.8	
10/23	98	117	287	2,596		1,287	1.047	57.1	
10/24	113	116	332	3,020		1,750	1.040	87.9	Plus 100 gm glucose
10/25	18	16	161	480	0.080	1,320		44.8	
10/26	127	142	341	3,147		950	1.050	47.0	
10/27	98	118	291	2,621		2,610	1.022	43.0	Fluid intake, 5,000 cc Fluid intake, 1,000 cc
10/28	87	101	282	2,368		1,250	1.045	59.6	
10/29	133	125	381	3,225		995	1.043	58.3	
10/30	182	127	374	3,488		1,840	1.034	57.5	Plus 100 gm glucose
10/31	168	51	221	2,016		1,000	1.042	61.7	
11/ 1	92	84	131	1,740		1,365	1.041	58.3	
11/ 2	76	88	91	1,460		1,000	1.038	41.7	Plus 100 gm glucose
11/ 3	147	65	223	2,073		1,220	1.043	75.0	
11/ 4	63	89	87	1,400		1,070	1.050	60.9	
11/ 5	87	55	93	1,215		850	1.045	51.8	Plus 100 gm glucose
11/ 6	102	47	163	1,571	0.076	1,060	1.044	59.9	
11/ 7	157	106	298	2,776		2,140	1.028	63.9	
11/ 8	99	74	189	1,821		1,195	1.042	62.9	Plus 100 gm glucose
11/ 9	101	65	89	1,353		2,411		53.6	
11/10	138	82	269	2,370					
11/11	122	105	189	2,289		725	1.045	51.8	Plus 100 gm glucose
11/12	102	145	259	2,846		1,055	1.048	74.2	
11/13	111	122	336	2,891		710	1.043	57.2	
11/14	89	101	291	2,429					Plus 100 gm glucose
11/15	101	141	336	3,018		1,365	1.050	98.9	
11/16	83	81	241	2,029		1,300	1.048	104.8	
11/17	91	77	262	2,092		1,794		72.4	Plus 100 gm glucose
11/18	112	116	321	2,774	0.090	875	1.045	56.8	
11/19	52	143	34.2	1,638		1,200	1.040	63.2	Low protein, low carbohydrate diet
11/20	47	179	35.9	1,873		1,130	1.039	50.0	
11/21	47	179	18.2	1,850		1,097	1.040	61.6	
11/22	41	176	34.4	1,889		1,265	1.040	67.9	Low protein, low carbohydrate diet
11/23	51	197	33.5	2,126		1,533	1.037	67.7	
11/24	55	190	35.5	2,078		1,127	1.043	58.3	
11/25	56	178	34.5	1,969		1,320	1.041	63.3	Low protein, low carbohydrate diet
11/26	52	169	35.9	1,878		1,150	1.045	82.2	
11/27	55	223	29.7	2,347		1,025	1.038	55.4	
11/28	56	171	31.8	1,884		1,195	1.041	66.4	Low protein, low carbohydrate diet
11/29	56	192	32.7	2,030		730	1.040	36.9	
11/30	55	204	41.1	2,226		1,030	1.042	56.6	
12/ 1	55	184	35.0	2,023		1,165	1.040	64.9	Low protein, low carbohydrate diet
12/ 2	57	223	29.7	2,353		850	1.033	31.8	
12/ 3	59	161	33.0	1,815		1,260	1.041	61.3	
12/ 4	56	216	32.4	2,297		1,380	1.036	52.8	Low protein, low carbohydrate diet
1/11	58	124	255	2,376		410	1.048	26.3	
1/12	55	115	235	2,195		1,145	1.046	74.2	Low protein, low carbohydrate diet
1/13	57	129	233	2,320		530	1.050	36.8	
1/14	58	143	256	2,540		360	1.033	19.8	
1/15	58	130	253	2,415		620	1.045	48.4	Low protein, low carbohydrate diet
1/16	57	144	253	2,545		390	1.044	27.5	
1/17	56	105	232	2,100		1,235	1.040	77.2	
1/18	48	169	234	2,190		520	1.045	36.6	Low protein, low carbohydrate diet
1/19	59	104	213	2,025		490	1.050	40.9	
1/20	56	114	196	2,035		365	1.040	15.7	
1/21	46	61	219	1,610		920	1.038	51.1	Low protein, low carbohydrate diet
1/22	49	128	187	2,095		705	1.043	44.1	
1/23	76	144	258	2,630		470	1.041	30.1	
1/24	46	120	234	2,200		2,330	1.037	122.6	100 gm glucose added to diet in divided doses
1/25	74	136	257	2,550		670	1.039	37.2	
1/26	63	127	248	2,385					
1/27	68	141	258	2,675		870	1.040	41.2	100 gm glucose added to diet in divided doses
1/28	65	128	267	2,380		820	1.039	38.7	
1/29	66	147	265	2,640		930	1.038	38.1	
1/30	72	133	254	2,400		780	1.039	28.9	100 gm glucose added to diet in divided doses
1/31	69	127	244	2,395	0.090	630	1.043	41.1	
2/ 3	0	0	0	0		1,200	1.033	72.3	Starvation
2/ 4	0	0	0	0		1,155	1.036	46.6	
2/ 5	0	0	0	0		970	1.042	61.4	
2/ 6	0	0	0	0					Starvation
2/ 7	0	0	0	0		875	1.032	39.3	
2/ 8	0	0	0	0		1,080	1.031	36.5	

was on the diet which we routinely give patients with diabetes mellitus to reduce their hyperglycemia, and which contains approximately 15 gm carbohydrate per day. From October 21 to November 18 her diet was not restricted, and her daily carbohydrate intake varied from 85 to 350 gm. During the third period, from November 19 to December 4, she was on a low protein, low carbohydrate diet with a daily carbohydrate intake varying less than 5 gm from 55 gm. From January 14 to January 21, she was on a diet containing about 240 gm of carbohydrate. During the following ten days the diet was not changed from that of the fourth period, except that 100 gm glucose were added daily, in three doses. Finally, she was starved for six days.

It is apparent that the amount of glucose in the urine had no relation to the amount of carbohydrate ingested, and that, while the variation in the glycosuria from day to day was large, it was not dependent on the diet. It is especially interesting to notice that the addition of 100 gm glucose to the intake did not increase the glycosuria, while complete starvation did not decrease it.

TABLE 2—RESPONSE OF BLOOD AND URINE TO 100 GM GLUCOSE BY MOUTH

Time	Water Ingested Cc	Blood Sugar per Cent	Urine					Average Normal Blood Sugar per Cent	Typical Diabetic Blood Sugar, per Cent
			Quantity, Cc	Specific Gravity	Glucose, Gm	Per Cent	Per Hour, Gm		
Fasting	400	0.071	74.0	1.044	4.1	5.5	2.3	0.078	0.11
15 minutes		0.087							
30 minutes	400	0.092	46.0	1.044	2.8	6.1	5.6	0.108	0.15
1 hour	200	0.090	65.5	1.040	4.1	6.4	8.2	0.093	0.24
2 hours	200	0.043	342.0	1.014	6.4	1.9	6.4	0.083	0.20
3 hours	200	0.048	523.0	1.008	4.6	0.9	4.6	0.078	0.20
5 hours		0.068	197.0	1.020	3.8	2.0	1.9		

Her reaction to ingested glucose was studied in both the urine and the blood. The patient was given 100 gm of glucose by mouth while she was in a fasting state in the morning. Sufficient water was given during the test to assure the collection of urinary specimens. The results are shown in Table 2. To the table, for convenience, is added a normal blood sugar curve, and a blood sugar curve of a diabetic, both taken from Strouse¹⁴. In this connection it will also be noted in Table 1 that the daily ingestion of 100 gm glucose added to the diet in divided doses had no effect on the amount of sugar in the urine.

The effect of diuresis was studied in three experiments. During one of the days when the patient was on an unrestricted diet, she was asked to drink 5,000 cc of fluid, on this day her sugar excretion was 43 gm. On the following day, her intake was limited to 1,000 cc, and her glucose output was 59.6 gm. This difference is within the limits of variation in her glycosuria when there was no apparent change in

¹⁴ Strouse, S. Alimentary Hyperglycemia. Arch. Int. Med. 26:759 (Dec.) 1920.

the conditions. On another occasion, while fasting, she was given water in the same amounts and at the same intervals as during the study of the effect of 100 gm glucose, that is, 400 c c at the start of the experiment, 400 c c at the end of thirty minutes, and 200 c c each at the end of the first, second and third hours. The results are presented in Table 3. It will be noted that the rate of glucose excretion remained practically constant, though the percentage of urinary glucose varied inversely with the diuresis. It will also be noted that the rate was practically the same as the average throughout her stay in the hospital, 60 gm a day is at the rate of 2.5 gm per hour. In the third experiment an effort was made to promote diuresis by oral administration of 90 grains theobromin sodium salicylate (diuretin) daily. This drug by mouth is also known to cause glycosuria.¹⁵ In this case, as in that of de Langen¹⁶ neither diuresis nor increase in the glucose excretion was noted.

TABLE 3—RESPONSE OF URINE TO WATER BY MOUTH

Time	Water Ingested, C c	Urine				
		Quantity, C c	Specific Gravity	Glucose, Gm	Per Cent	Per Hour, Gm
Fasting	400					
30 minutes	400	108.0	1.041	6.8	6.3	2.4
1 hour	200	24.0	1.040	1.3	5.4	2.6
2 hours	200	215.0	1.010	2.2	1.0	2.2
3 hours	200	366.0	1.010	2.7	0.7	2.7
5 hours		72.0	1.034	3.3	4.6	1.7

From the time when Klemperer¹⁷ first declared the existence of renal glycosuria, much interest has been centered on the state of the kidney function in the cases. Klemperer insisted that it is characteristic of the affection that when nephritis develops, the glycosuria disappears. Other observers, on the contrary, have believed that renal glycosuria is a part of nephritis. Luthje¹⁸ claimed such a relationship, because his patient was apparently free from sugar before the development of nephritis, and developed glycosuria shortly after nephritis. Naunyn¹⁹ also upheld this view, and reported three cases in nephritics, there were no blood sugar determinations in his data, however, and the diagnosis of renal glycosuria is not established. Tauchau's patient³

15 Nishi, M. Ueber den Mechanismus der Diuretinglycosurie, Arch. f. exper. Path. u. Pharmacol. **61** 401, 1909

16 de Langen, C. D. Beitrag zur Kasuistik des renalen Diabetes, Berl. klin. Wchnschr. **51** 1792, 1914

17 Klemperer, G. Ueber regulatorische Glycosuria und renalen Diabetes, Berl. klin. Wchnschr. **33** 571, 1896

18 Luthje, H. Beitrag zur Frage des renalen Diabetes. Munchen med. Wchnschr. **38** 1471, 1901

19 Naunyn, B. Der Diabetes Mellitus, Wien, 1906, p. 136

had a transient acute nephritis. Both of the cases reported by Bailey⁶ gave evidence of nephritis. In the majority of the cases, however, renal function was not definitely impaired.

The urine of our patient frequently showed a trace of albumin, but casts were found at no time. The urea content of her blood was determined on two occasions, during the period when she was on an unrestricted diet, and found to be 36 and 40.8 mg per 100 cc, respectively. Phenolsulphonephthalein, injected intramuscularly, was recovered in the urine to the extent of 65 per cent in the first hour and 20 per cent in the second, on repetition several days later, 63 and 14 per cent, respectively, were recovered. No evidence of nephritis could be obtained.

The nitrogen determinations on the urine of the starvation period raise some interesting but unanswerable questions. The values given were determined by Folin's micro-Kjeldahl method and checked by the original Kjeldahl method. These figures, which are presented in

TABLE 4—URINARY GLUCOSE AND NITROGEN DURING STARVATION

Date	Glucose, Gm	Nitrogen, Gm	D	N
2/3	72.3			
2/4	46.6	6.352	7.33	1
2/5	61.4	4.268	14.38	1
2/6				
2/7	39.3	3.313	11.86	1
2/8	36.5	4.536	8.05	1
Average	45.9	4.61	9.96	1

Table 4, gave a D/N ratio of about 10/1. It seems safe to assume that on the sixth day of starvation the glycogen deposits of the body have been exhausted, and we have no evidence in support of the view that fat is converted into glucose. Yet it is hard to believe that all this sugar was derived from protein.

December 9, she complained of diffuse abdominal pain. She began vomiting and all efforts toward moving her bowels failed. Her abdomen was rigid throughout. A diagnosis was made of intestinal obstruction and she was transferred to the Surgical Clinic for operation. At operation, adhesions were found about her colon, with a sharp kinking of the intestine. Her uterus, tubes and right ovary were found to have been removed, the left ovary was in place. The adhesions were separated and the intestine freed. She returned from the operating room in good condition and made an uneventful recovery. The wound healed rapidly, and there were never any symptoms of acidosis.

SUMMARY

A case of renal glycosuria is described. During a period of four months the patient excreted an average of 60 gm glucose daily with

a variation of from 15 to 122 gm. The amount was not related to the carbohydrate intake. After the ingestion of 100 gm. glucose, the blood sugar rose from a fasting per cent. of 0.071 to 0.092 in thirty minutes, by the end of two hours it had fallen to 0.043 per cent, and after five hours it was found to be 0.068 per cent. Even with a blood sugar as low as 0.043 per cent., glucose was excreted at the rate of over 5 grams per hour. Diuresis by increased water ingestion caused no increase in the glycosuria, and no effect was seen from the administration of diuretic in large doses. No evidence of nephritis was found. The D/N ratio in the urine of starvation was about 10/1. She reacted to a laparotomy for acute intestinal obstruction in a perfectly normal manner.

I wish to thank Dr. L. H. Newburgh for his kind and helpful suggestions in this study.

THE RIGHT VENTRICLE IN PULMONARY TUBERCULOSIS *

ERNST P BOAS, M D, AND HUBERT MANN, M D
NEW YORK

Current medical opinion, in so far as it is directed to the heart in the tuberculous patient, seems to take it for granted that in chronic phthisis there is frequently found a dilatation and hypertrophy of the right ventricle. This is explained usually by the assumption that with extensive pulmonary disease there is a partial obliteration and obstruction of the pulmonary stream bed which exacts an increased amount of work from the right ventricle to maintain the circulation. This mechanism would be analogous to the left ventricular hypertrophy that accompanies arterial hypertension. Thus Bohland¹ states that such a hypertrophy is present in chronic phthisis, that it is compensatory in its nature, and that the enlarged right ventricle, having little reserve, easily becomes insufficient. Kiehl,² too, mentions that such hypertrophy is of frequent occurrence.

On analysis, the evidence for this belief is found to be conflicting and for the most part inadequate. Portal³ in 1792, concluded, on the basis of necropsy studies, that the right auricle and ventricle dilate in chronic phthisis because of obstruction of the pulmonary blood flow. Laennec, Grissolle, Louis, Rokitansky and Rigal, on the other hand, state that dilatation of the right heart in tuberculosis occurs only exceptionally. Potain⁴ noted that the heart of the phthisical patient was usually small, and believed that this was due to the cachexia which accompanies the disease. In those patients in whom the progress of the emaciation was very slow, however, large hearts were often found, which enlargement, Potain thought, was usually due to extrapulmonary causes. Regnault,⁵ too, found a true or an apparent hypertrophy of the heart in the majority of cases of fibroid phthisis. He believed that it was caused by complications which acted directly on the heart and delayed the evolution of the tuberculosis with its accompanying cachexia. Dilatation of the right heart, particularly of the auricle, he claimed could be demonstrated quite frequently among

¹From the Montefiore Hospital for Chronic Diseases

* Read before the Section of Medicine of the New York Academy of Medicine, Feb 15, 1921

¹ Bohland, K. Handbuch der Tuberkulose First Half 4 p 5

² Krehl, L. Pathologische Physiologie, Ed 7, p 30

³ Portal, A. Observations sur la Nature et le Traitement de la phthisie pulmonaire, Paris, 1792

⁴ Potain. Le Cœur des Phthisiques 1892

⁵ Regnault, E. Le Cœur chez les Tuberculeux, Paris 1899

living tuberculous patients by percussion, but more rarely was this shown at necropsy. Regnault believed that emphysema, pleural adhesions, sclerosis and caseation of the lungs, all tending to obstruct the pulmonary blood bed were the cause of this hypertrophy. A number of other authors have arrived at similar conclusions, although they have not clearly differentiated hypertrophy from dilatation. Among these may be mentioned Bard, Decroix, Maruchau, Fraentzel and Buhl⁶.

Two physiologic studies throw some light on this question. In 1876 Lichtheim⁷ published an excellent monograph on the physiology of the pulmonary circulation. A brief summary of his conclusions will be illuminating. He first discusses the conventional views on the disturbances of the pulmonary circulation. These had been based on the assumption that a partial obstruction of the lesser circulation, by narrowing the blood bed, prevented the right ventricle from propelling to the left ventricle the same amount of blood as before. Under such circumstances, the left ventricle received an inadequate amount of blood, the arterial blood pressure fell, and the right ventricle became over-filled. To establish equilibrium, the right ventricle had to work harder and eventually hypertrophy. Then the velocity flow in the pulmonary circulation rose until the amount of blood passing through the pulmonary stream bed per unit of time was normal. On this basis were explained the venous stasis, the small pulse, the low blood pressure and oliguria found in pulmonary embolism and in acute pleural effusions. Lichtheim attempted to verify experimentally this theoretical explanation of the mechanism of cardiac response to disturbances of the pulmonary circulation. He found that ligation of the left pulmonary artery had no effect on the arterial blood pressure nor on the heart rate, and that a variation in the carotid pressure did not affect the pulmonic pressure. His final conclusions, drawn from a long series of carefully executed experiments, are: 1 After closure of parts of the pulmonary stream bed the total amount of blood that courses through the remaining vessels is the same as formerly passed through the intact pulmonary circulation. 2 This is accomplished by a rise in pressure in the remaining, i. e., open, pulmonary arteries, together with an increased velocity of flow and a stretching of the arterioles. 3 This mechanism adequately maintains the circulation if not more than three quarters of

6 Bard. These de Lyon, 1879. De la phthisie fibreuse chronique. Decroix. Atrophie du cœur et dilatation des cavités droites dans la tuberculose pulmonaire, These de Paris, 1880. Maruchau. Etat du cœur droit dans la phthisie pulmonaire, Thèse de Paris, 1871. Fraentzel, O. The Idiopathic Enlargements of the Heart, Wood's M. & S. Monographs, May, 1890, p. 523. Buhl. Messungen der Herzventrikel und der grossen Gefässe, Stuttgart, 1878.

7 Lichtheim, L. Die Störungen des Lungenkreislaufs und ihr Einfluss auf den Blutdruck, Berlin, 1876. (Hirschwald.)

the pulmonary blood bed is shut off. If the closure exceeds this figure, in spite of the high pulmonic pressure the blood supply to the left heart fails and the arterial pressure drops. The effect that this compensatory process will have on the right ventricle will vary with the extent of the narrowing of the stream bed of the lungs. At first, the dilatation of the arterioles will offset the narrowed blood bed, but with more extensive obstruction of the pulmonary vessels, the right ventricle will be called on for more work to maintain the minute volume flow in the lungs, and will dilate and eventually hypertrophy. The rise in pressure in the pulmonary artery clearly indicates that the right chamber of the heart is working under a greater load.

There are still other factors which determine the effect that diseases of the lung will have on the heart. The most important of these is the elasticity of the lungs which is ably discussed by Baumler⁸. He recalls that because of the elasticity of the lungs the external pressure on the heart and blood vessels of the mediastinum is less than atmospheric. Within the lungs, and in the rest of the body, however, the pressure is equal to one atmosphere. Thus the flow of the venous blood into the heart, and of the pulmonary blood into the left auricle is aided by this difference of pressures. Extensive pleural adhesions, he says, impair the elasticity of the lungs and prevent the operation of this factor, and are as potent as obstruction of the pulmonary blood bed in increasing the work of the right ventricle and in inducing hypertrophy. It must be remembered, however, that while this negative intrathoracic pressure aids the flow of blood from the lungs to the left auricle, it hinders the flow of blood from the right ventricle to the lungs to exactly the same degree, and that, therefore, a change in this intrathoracic pressure will not alter the total work done by either chamber of the heart. Another element must, however, be taken into consideration. The increased amount of connective tissue which is found in the lung with massive adhesions will impair the elasticity of the lung tissue and will have an effect on the pulmonary vessels which may be analogous to arteriosclerosis in the larger circulation. Such an indirect impairment of the elasticity of the pulmonary vessels will increase the work of the right heart.

The clinical experience of a number of men gives support to this theory. Morgagni⁹ described cases in which the necropsy revealed pleural adhesions associated with hypertrophy of the right heart.

⁸ Baumler, Ch. Ueber Obliteration der Pleurasacke und Verlust der Lungenelasticität als Ursache von Herzhypertrophie, *Deutsch Arch f klin. Med* 19 471

⁹ Morgagni. Quoted from Grau-Ronsdorf, *Internat Centralbl f Tuberkuloseforschung* 5 283, 1910

Traube,¹⁰ in 1866, presented several necropsy protocols of patients with extensive pleural adhesions and secondary hypertrophy of the right ventricle. Du Castel,¹¹ in a study of fifteen cases of tuberculosis, found right ventricular hypertrophy only if pleural adhesions or pulmonary sclerosis were present. Palhier¹² describes cases in which the post-mortem examination showed dilatation of the right heart, associated with pleural adhesions and emphysema, and it is to these factors, rather than to a restriction of the blood bed that he attributes the change. On the other hand, Lienart,¹³ who reports dilatation of the right heart in two thirds of his cases of phthisis, ascribes the cause to a diminution of the blood bed from fibrosis and emphysema. Sokolowski¹⁴ cites several necropsies of patients with fibroid phthisis in whom he found enlarged right hearts.

All of these clinical and pathologic reports are open to the criticism that the methods by means of which the cardiac hypertrophy was determined are not sufficiently accurate. Exact pathologic recognition of hypertrophy of one or both chambers of the heart demands a painstaking dissection and weighing of the organ according to the method of Mueller. A search through the literature reveals only three investigators who have employed this technic in the study of the heart of the tuberculous. Hirsch¹⁵ studied 120 cases of tuberculosis, in 35 per cent of which he discovered a marked right ventricular hypertrophy. The degree of hypertrophy paralleled the extent of pleural adhesions and of induration of the lung. In acute ulcerative tuberculosis, the heart was small and atrophied. Wideroe¹⁶ found that right ventricular hypertrophy was quite frequent in chronic pulmonary tuberculosis, and that it was the greater, the greater the age of the individual, or the more extensive the lesion. Bret¹⁷ arrived at similar conclusions, but pointed out that the degree of hypertrophy did not always parallel the extent of the lung lesion, nor the duration of the illness.

In view of these findings reported in the literature, and of theoretical considerations which suggested that there was some physiologic basis for the changes that had been described, an electrocardiographic study of a series of tuberculous patients was made in the hope that by this

10 Traube, L. *Gesammelte Beitr z Path u Physiol* **3** 338, 1866

11 Du Castel. *Recherches sur l'hypertrophie et la dilatation des ventricules du cœur*, *Arch Gen de med*, 7 ser **5** 25, 1880

12 Palhier. *Contribution a l'etude anatomopath du cœur dans la phthisis chronique*, *These de Paris*, 1890

13 Lienart. *Dilatation du cœur droit chez les tuberculeux*, *Thèse de Paris*, 1886

14 Sokolowski, A. *Ueber die fibrose Form der Schwindsucht*, *Deutsch Arch f klin Med* **27** 443, 1885

15 Hirsch, V. *Ueber die Beziehung Zwischen Herzmuskel und Korpermuskulatur*, *Deutsch Arch f klin Med* **68** 337, 1900

16 Wideroe, S. *Die Massenverhältnisse des Herzens unter pathologischer Zuständen* Christiania, 1911, abstr from *Zentralbl f Herz u Gefasskrankheiten* **3** 121, 294, 1911

17 Bret, J. *Lyon med* **122** 452, 1914

means we might be able to determine whether or not there was a predominance of one of the chambers of the heart. At the same time, the case was studied in order to correlate the electrocardiographic with the clinical findings. The material from which this study was made consists of ninety-seven consecutive, unselected cases from the tuberculosis service of the Montefiore Hospital.¹⁸

The procedure of examination was as follows. The salient points in the history were noted, and then the patient was subjected to a physical examination in which especial attention was directed to the cardiovascular system. The blood pressure was taken, the pulmonary lesion was noted, particularly whether or not it was of the fibroid type, and the degree of activity of the lesion was remarked. Electrocardiograms of the patients were taken in the usual way with a Cambridge electrocardiograph and immersion electrodes. The conventional three leads were taken, and the electrocardiograms were interpreted by one of us (Mann) who had no clinical knowledge of the case. Roentgenograms were made of many patients, but they were of little assistance in this study. With extensive lung lesions, the outlines of the borders of the heart are often obscured, and cardiac displacement is very frequent. This makes it very difficult to decide from a plate whether or not there is a slight enlargement of one of the ventricles.

The results do not confirm the views which we held at the outset of this study, but present a number of interesting points. The first fact that stands out is that in relatively few cases is predominance of one chamber or another very marked. The reports usually read, "tendency to left ventricular predominance" or "slight right ventricular predominance." Of the ninety-seven cases, twenty-eight show right, twenty-nine left ventricular predominance, and in forty there was no predominance of either ventricle. Three of the patients exhibiting left ventricular predominance must be excluded from the series because they show definite extrapulmonary causes for left ventricular hypertrophy. Even without them, the frequency of occurrence of right and left ventricular predominance is about equal. An analysis of the clinical features of the cases was made in the attempt to explain this phenomenon.

It is well known that many tuberculous patients give evidence of a mild hyperthyroidism, particularly early in the course of the disease. This is manifested by thyroid enlargement, slight prominence of the eyes, frequently associated with a positive Von Graefe sign, nervousness, tachycardia and vasomotor disorders. With the idea that the left ventricular predominance might be caused by such a thyroid disorder, the patients were grouped into those giving definite symptoms of

18 We are indebted to Dr. Fishberg, director of the service, for permission to study the patients.

thyroid disturbance and those in whom the symptoms were suggestive. Another group was made up of the fibroid cases, and, finally, patients were classed according to the degree of activity of the tuberculous lesion. Table 1 presents the data.

TABLE 1—VENTRICULAR PREDOMINANCE OF VARIOUS GROUPS OF CASES

	R V P	L V P	Normal*
Definite hyperthyroidism	6	8	9
Possible hyperthyroidism	9	10	9
Fibroid phthisis	10	13	16
Active cases	13	15	22
Inactive cases	15	12	18

* Normal means predominance of neither chamber.

In two cases showing L V P, the activity of the lesion was not noted.

These figures give no evidence of correlation between predominance of one ventricle and any of the factors mentioned in the table. But it is possible that in each case several influences may be at play, and that the final effect is the resultant of these components. This suggests a study of some of the individual cases.

Those with fibroid phthisis may be first considered.

REPORT OF CASES

CASE 1 (38893)—J B, male, aged 32, duration, seven years. Lesion extensive bilateral fibroid phthisis, inactive. Heart, normal to physical examination. Pulse, full, soft, rate 84. Blood pressure, 95/70. Veins of right neck rather full. No signs of hyperthyroidism. Electrocardiogram, right ventricular predominance.

This case taken by itself shows a definite right ventricular predominance conditioned apparently by a fibrosis of the lung. That the situation is not so simple is shown by Cases 2 and 3.

CASE 2 (44142)—N K, male, aged 39, duration, one year. Lesion bilateral cavitation and fibrosis, associated with asthmatic attacks, active. Heart normal to physical examination. Pulse, weak, rate 96. Blood pressure, 106/65. Slight cyanosis. Thyroid not felt, slight Von Graefe sign present. Electrocardiogram, left ventricular predominance.

CASE 3 (38146)—E B, female, aged 32, duration, four years. Lesion fibroid phthisis of both upper lobes with asthmatic breathing all over chest, active. Heart normal to physical examination. Pulse, small, soft, rate 124. Blood pressure, 120/74. Slight cyanosis. No signs of hyperthyroidism. Roentgenogram, heart abnormally small. Electrocardiogram, normal.

Clinically these two cases correspond to Case 1, yet in one of them there is left ventricular predominance, and in the other the heart is normal. The cause for this variation is not apparent.

A similar series may be shown to exist in the thyroid cases.

CASE 4 (41424)—B K, female, aged 22, duration, six months. Lesion consolidation of the whole right lung, active. Heart small. Roentgenogram, heart abnormally small. Pulse, good quality, rate 140. Blood pressure, 125/70.

Thyroid, moderate general enlargement, von Graefe sign present Electrocardiogram, left ventricular predominance

CASE 5 (41364) —E L, female, aged 19, duration, six months Lesion infiltration of whole left lung, infiltration with multiple cavities of right upper lobe, active Heart, displaced a bit to the left Systolic murmur at pulmonic area Pulse, good, rate 132 Blood pressure, 108/78 Thyroid isthmus palpable, von Graefe sign present Roentgenogram, abnormally small heart displaced to the left Electrocardiogram, right ventricular predominance, not well marked

CASE 6 (39806) —E K, female, aged 25, duration, two years Lesion cavitation and infiltration of left upper lobe, left artificial pneumothorax, infiltration of right upper lobe, active Heart displaced to left Pulse, small, weak, rate, 108 Blood pressure, 104/74 Thyroid enlarged Von Graefe present Patient is very unstable emotionally Electrocardiogram, normal

Here, again, are three cases, almost identical clinically, but presenting absolutely different electrocardiographic findings

Since the electrocardiogram does not give information as to whether or not hypertrophy has occurred in one or the other of the ventricles, but merely indicates the predominance of one chamber over the other, depending on the relative amounts of their muscle mass, the thought occurs that several factors may be operative in some of these patients, that, for instance, in a case showing both fibrosis and hyperthyroidism, both chambers of the heart may hypertrophy, but their relative muscle mass may remain unchanged The following cases could be cited in support of this proposition

CASE 7 (39121) —W S, male, aged 53, duration, five years Lesion bilateral extensive fibroid phthisis, inactive Heart, normal to physical examination Pulse, good, rate, 104 Blood pressure, 170/95 Slight edema of feet, marked myocardial insufficiency some months ago Urine shows albumin and casts No signs of thyroid disorder Electrocardiogram, normal

CASE 8 (41127) —S L, male, aged 32, duration, two years Lesion extensive bilateral fibrosis, inactive Heart displaced slightly to the left Pulse, good, rate, 104 Thyroid isthmus enlarged, von Graefe sign present Nervous instability Blood pressure, 104/68 Electrocardiogram, normal

The interplay of the hypertension and fibrosis in the first instance, and of the slight hyperthyroidism and fibrosis in the second, might account for the normal electrocardiographic complex But a further study of many individual cases does not bear this out The different electrocardiographic findings seem to be distributed quite vicariously, and we have been unable to determine any clinical criteria from which we might venture to predict whether there was predominance of either of the ventricles The activity or inactivity of the pulmonary lesion is not a factor, nor has the duration of the illness any influence (Table 3) If, however, we classify the cases according to sex, an interesting relationship appears (Table 2)

Although the series is not large, the differences are too great to be accidental. To make sure of this point, these and other data were checked up statistically. The striking fact appears that left ventricular predominance is about twice as common in the women as in the men, and that right ventricular predominance is twice as frequent in the men as in the women. It is difficult to find a satisfactory explanation of

TABLE 2—CLASSIFICATION OF CASES ACCORDING TO SEX

Sex	L V P	R V P	Normal	Total
Female	19	9	20	48
Male	10	20	19	49

this phenomenon. It is suggestive, however, that symptoms of hyperthyroidism were found more than twice as often in the women as in the men. The pulse rates, too, are interesting. Of the forty-eight women, thirty-one had a pulse rate over 110. Of the forty-nine men, only ten had a pulse rate over 110, and in sixteen it was below 90.

A further analysis is detailed in Table 3.

The difference in the ages of the two sexes is accidental. Probably for social and economic reasons, the older women do not enter our hospital so frequently as do the older men. But the significant fact is that in spite of the disparity of the ages in the male and female series, the age distribution of predominance of the ventricles runs parallel in both. Thus, in both sexes right ventricular predominance is found in the youngest, no predominance in the next age group, and left ventricular in the oldest. We have been unable to discover whether this has any particular significance. Such a tendency to predominance of

TABLE 3—DETAILED ANALYSIS OF CASES

	Male			Female		
	Number of Cases	Average Age, Years	Average Duration, Years	Number of Cases	Average Age, Years	Average Duration, Years
R V P	20	39.0	5.9	9	23.6	2
Normal	19	44.8	4.8	20	29.2	4
L V P	10	48.2	5.2	19	36.5	3.3

the left chamber is, of course, more frequent in the older age groups. It will be noted that the duration of the tuberculosis has no influence on the distribution.

Another fact resulting from this study is that displacement of the heart, which is so common in the tuberculous, and which is often extreme, ordinarily has no effect on the outline of the electrocardiogram.

SUMMARY

1. An electrocardiographic study of ninety-seven patients with pulmonary tuberculosis revealed 29 per cent with right ventricular pre-

dominance, 30 per cent with left ventricular predominance, and 41 per cent with no predominance of either chamber

2 Right ventricular predominance is not always associated with any particular type of tuberculosis. It is found more commonly in young than in old patients, and twice as frequently in men as in women.

3 Left ventricular predominance is not always associated with any particular type of tuberculosis. It is found more commonly in older patients, and is twice as frequent in women as in men.

4 Left ventricular predominance occurs more frequently with the increasing age of the patient.

5 In contrast to the necropsy findings reported in the literature, right ventricular predominance is not found more frequently in association with fibroid phthisis, or with pleural adhesions, than with other types of pulmonary tuberculosis.

FREQUENT CAUSES AND THE TREATMENT OF SEASONAL HAY-FEVER *

I CHANDLER WALKER, M D

BOSTON

Although John Bostock,¹ in 1819, first described the symptom complex of hay-fever, it was not until 1873 that Blackley² determined the real cause of the symptoms, namely, the pollen of plants. Curtis,³ in 1900, was the first to attempt to produce active immunity or to treat the condition, he used extracts of the whole plant. Since Dunbar,⁴ in 1905, was the first to employ extracts of the plant pollen, the modern treatment of hay-fever was really begun by him. During the past ten years reports on the treatment of hay-fever have been too numerous to justify reference to all of them in this paper, but the work of Noon, Freeman, Koessler, Cooke, Scheppegegrell, Goodale, Selfridge and possibly of others justify the mention of their names since it is these who have done most toward establishing the treatment of seasonal hay-fever.

Notwithstanding the number of papers on the subject, there is a paucity of specificity as regards the pollens that actually cause hay-fever, and, likewise, the treatment of the condition has been stated too often in a general way. As a result, there would seem to be as many different pollens actually causing hay-fever and as many different ways of treating hay-fever as there have been investigators. A natural result was that commercial houses have put on the market pollen preparations consisting of mixtures of the various pollens that prevailed at definite seasons, and these mixtures were used by the medical profession all over the country. In other words, neither the physician nor the commercial house have been concerned as to whether any particular pollen was indigenous and caused hay-fever in one locality to the exclusion of other pollens in other localities, and the physician would not or could not determine by tests which of the prevailing pollens was the actual cause of symptoms, the pollen mixtures were used hit or miss. From the practical standpoint, the results from such treatment could not be as satisfactory as would be the case if the

* From the medical clinic of the Peter Bent Brigham Hospital

1 Bostock, J. Case of Periodical Affection of the Eyes and Chest, *Med Chir Tr*, Lond **10** 161, 1819

2 Blackley, C H. Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hay-Fever or Hay Asthma). London **7** 202, 1873

3 Curtis, H H. The Immunizing Cure of Hay Fever, *Med News* **77** 16 (July 7) 1900

4 Dunbar, W P. *Berl klin Wchnschr* **17** 797, 877, 915, 942, 1237, 1905, *Deutsch med Wchnschr* **32** 578, 1911

patient were treated by the pollens to which he was actually exposed and to which he was most sensitive. Therefore, the excuse for publishing the present paper in considerable detail is to help clarify the present status of the treatment of seasonal hay-fever as regards patients living in the New England States, and it is hoped that other investigators in the same and in other localities will be stimulated to detail their investigations. It is only by such detailed reports that it will be possible to learn the prevailing causative pollens in various localities and the best method of treatment.

After a description of the methods used by me in testing and treating hay-fever patients, this paper will consist of a series of tables. Table 1 presents those patients who were treated with ragweed pollen only one year, Table 2, patients treated with grass pollen only one year, Table 3, patients who had insufficient treatment with ragweed and with grass pollen, Table 4, patients treated two years in succession with ragweed pollen, Table 5, patients treated two years in succession with grass pollen, Table 6, patients treated three successive years with ragweed pollen, Table 7, those treated three successive years with grass pollen, and Table 8, those treated four successive years with ragweed pollen. In all of these tables the patients were treated preseasonally, thereby attempting to prevent symptoms. Table 9 presents patients who were treated during the season with grass pollen, Table 10, those treated during the season with ragweed pollen, and Table 11, patients treated during the season with bacterial vaccines. In these tables the patients were treated during the season, thereby attempting to relieve symptoms. Table 12 presents patients treated both preceding and during the season. The pollens of trees and pollens other than those already mentioned will be discussed as possible causes of hay-fever. Finally, other parts of plants, animal emanations, foods, bacteria and olfactory irritants will be discussed as causes of seasonal hay-fever.

The cutaneous or skin test, which has proven satisfactory to me,⁵ was employed to determine the sensitivity of the patients to the various pollens. A number of small cuts, each about an eighth of an inch long, are made on the flexor surfaces of the forearm. These cuts are made with a sharp scalpel, but are not deep enough to draw blood, although they do penetrate the skin. On each cut is placed a pollen and to it is added a drop of tenth normal sodium hydroxid solution to dissolve the pollen protein and to permit of its rapid absorption. Instead of using the whole pollen, a concentrated solution of pollen protein or extract may be used without the addition of sodium hydroxid. At the end of half an hour the pollens are washed off and the reactions are

5 Walker, I. C., and Adkinson, J. A Comparison Between the Cutaneous and the Intradermal Tests in the Sensitization of Asthmatic and Hay Fever Patients, *J. M. Research* **37** 287, 1917.

noted, always comparing the inoculated cuts with normal controls on which no pollen was placed. A positive reaction consists of a raised white elevation or urticarial wheal surrounding the cut. The smallest reaction that we consider positive must measure 0.5 cm. in diameter. All larger reactions are noted by a series of plus marks, any smaller reaction is called doubtful. The method of obtaining pollen from the flower has been outlined in Study 11.⁶

Having determined by these tests which pollens give a positive reaction, before a patient can be treated with the pollen it is necessary to know how sensitive that patient is to the pollen, therefore, different strengths of solutions of the pollen protein are tested in a similar manner. These solutions are made as follows. To 0.5 gm. of the dry pollen is added 44 c.c. of sterile physiologic sodium chlorid solution, and the mixture is shaken thoroughly at frequent intervals for twenty-four hours, after which enough absolute alcohol (6 c.c.) is added to the mixture to make the alcoholic content 12 per cent. Again, the mixture is thoroughly shaken at frequent intervals for twenty-four hours, after which it is centrifugalized at high speed and the supernatant fluid is pipetted off and saved. This supernatant fluid, therefore, consists of the pollen protein dissolved in a 12 per cent alcoholic physiologic sodium chlorid solution and it represents, by weight, 1 part pollen to 100 parts solvent. This 1:100 solution is used as stock, and from it other dilutions, 1:500, 1:1,000, 1:5,000 and 1:10,000 are made, using a 12 per cent alcoholic physiologic sodium chlorid solution as a diluent. These solutions are used not only for the skin tests but for treatment, and with the addition of a small crystal of thymol they keep for many months in a cool place.

Method of Treating Preseasonally with the Pollen Extracts Follows—The first treatment consists of from 0.1 to 0.2 c.c. of that dilution next higher than the one which gave a positive skin test, or, in other words, the first dose is 0.1 c.c. or 0.2 c.c. of the strongest dilution which failed to give any skin reaction whatever, no matter how slight. With our pollen extracts the majority of patients whom we treated gave a more or less positive reaction with the 1:5,000 dilution, therefore, the first treatment consisted of 0.1 c.c. or 0.2 c.c. of the 1:10,000 dilution. Treatments were given subcutaneously once a week, and each week the amount of the extract was gradually increased, so that as the treatment progressed, stronger and stronger dilutions were used, until one or more doses of the 1:100 dilution were given. As an example, I will give what I have found by experimentation to be the best outline of treatment for a patient who gives a more or less positive skin test with a 1:5,000 dilution of pollen extract, 1:10,000, give 0.15 c.c., 1:5,000.

⁶ Walker, I. C. Studies on the Sensitization of Patients with Bronchial Asthma to the Various Pollens, J. M. Research **36** 237, 1917

give 0.15 cc, 0.25 cc, 0.35 cc, 0.45 cc, 1:1,000, give 0.15 cc, 0.25 cc, 1:500, give 0.15 cc, 0.25 cc, 0.35 cc, 0.45 cc, 1:100, give 0.15 cc, 0.2 cc, 0.25 cc. Each dose was given preferably at weekly intervals and never oftener than once every five days.

This schedule of treatment calls for fourteen inoculations, however, for some reason or other, modifications frequently have to be used. An occasional patient is so sensitive to the pollen that a 1:10,000 dilution gives a slight reaction, thus necessitating an initial dose of 0.15 cc of 1:20,000 followed by possibly two doses of 1:10,000. Often it happens that a patient has considerable local or general reaction following some one treatment in the schedule, thus necessitating the repetition of that particular dose before the next increase may be given. More often the patient presents himself for treatment too late to complete the scheduled series of treatments before the onset of pollination so that for preseasonal treatment alone, some of the final treatments in the schedule must be omitted. This schedule is often modified purposely with certain individual cases. For instance, in some cases the second treatment with the 1:1,000 dilution, namely, 0.25 cc, is omitted, and in some cases instead of giving 0.15 cc of the 1:100 dilution, when this happens to be the final treatment that the patient is to receive because of onset of pollination, a fifth treatment with the 1:500 dilution, namely, 0.55 cc, is often substituted, and even a sixth treatment with the 1:500 dilution, namely, 0.65 cc, is sometimes given. These larger doses of 1:500 approximate the amount of protein in 0.15 and 0.2 cc of the 1:100 dilution, therefore, the fifth and sixth treatment with the 1:500 dilution, as outlined, is practically the equivalent of giving 0.15 cc and 0.2 cc of the 1:100 dilution. Since by far the great majority of patients are treated from three to five times with the 1:500 dilution, and since this number of treatments has given fairly satisfactory results, I consider this number of treatments, which consists usually of a total of ten, as worth giving, although a continuance of the schedule beyond three doses of the 1:500 dilution is most desirable, and giving less than three treatments with the 1:500 dilution is undesirable. Tables 1 and 2 will illustrate the results obtained from giving three or more treatments with the 1:500 dilution, and Table 3 will illustrate the results obtained from giving less than three treatments with the 1:500 dilution.

Since in the New England States the majority of patients have the first hay-fever symptoms between Aug. 10 and 20, during which time the compositae, chiefly ragweed, begin to pollinate, in order to complete the above schedule just previous to the onset of symptoms and pollination, patients must begin treatment between the last week in April and the first two weeks in May. Beginning treatment the first week in June will permit of giving from three to five treatments with the 1:500

dilution Likewise, since the early type of hay-fever, or so-called rose cold, which is usually caused by the grasses, begins the last few days of May or the first of June, treatment for this type of hay-fever should begin about the first of March, and the starting of treatment as late as the first of April will not permit of more than three or four treatments with the 1 500 dilution, according to the schedule outlined Naturally, in localities outside of New England, these seasons would differ, and the beginning of treatment consequently would vary, also the causative pollen must differ

Before Table 1 may be discussed, attention should be called to the following facts In the New England States ragweed (*ambrosia artemisiifolia*, or dwarf ragweed) is not the only plant that pollinates during August and September, nor is it the only pollen to which individuals are exposed or with which patients were tested Most of the compositae, such as golden rod, sunflower, golden glow and aster, pollinate during August and September, daisy pollinates during June and July, and dandelion in the early spring, giant ragweed is rarely encountered in the New England States In order to simplify the table, and because in my experience pollens other than ragweed rarely, if ever, are the chief cause of symptoms during the late hay-fever season, only ragweed is mentioned in the table although tests were made with the other pollens During the years 1917, 1918 and 1919 all of the patients in Table 1 were tested with golden rod and daisy, and the majority were tested with golden glow and sunflower During 1920, many of the patients were tested with these pollens One hundred and twenty patients in Table 1 were tested with the pollens of daisy and golden rod, and 100 were also tested with the pollen of sunflower and golden glow Fifty per cent of those tested with golden glow and with sunflower gave a positive reaction with the whole pollen, but none of these gave a reaction with a 1 100 dilution of the pollen, the other 50 per cent failed to react with the whole pollen Therefore, it may safely be stated that the pollens of golden glow and sunflower are not primarily causes of hay-fever in New England Of those tested with daisy pollen, 65.5 per cent failed to react to a 1 100 dilution of daisy pollen, 21 per cent did react to the whole pollen but not to a 1 500 dilution, 10 per cent reacted more or less positively to a 1 500 dilution of the pollen, and only 3.5 per cent reacted to as high a dilution as 1 1,000 Therefore, in comparison with ragweed, in only 3.5 per cent could one consider that daisy might be a cause of hay-fever as judged by tests, but it should be borne in mind that daisy pollinates during June and July and has finished pollination before ragweed begins, therefore daisy cannot complicate the causes of August and September hay-fever Of the 120 patients in Table 1 who were tested with golden rod pollen, 78.5 per cent failed to react with a 1 100 dilution of the pollen, 10 per cent

TABLE 1—PRESEASONAL TESTS AND TREATMENT WITH RAGWEED POLLEN

Patient	Age of Onset	Duration	Tests Before Treatment	Number of Treatments With Final Dilution	Tests at End of Treatment	Result of Treatment*	Patient	Age of Onset	Duration	Tests Before Treatment	Number of Treatments With Final Dilution	Tests at End of Treatment	Result of Treatment
			Season 1917										
1	42	25	1-1,000±	1-100 4	1-100+	Good	97	9	8	1-10,000±	Continued		Fair
2	8	7	1-5,000±	1-100 2	1-100+	Good	98	37	3	1-10,000±	1-500 4	1-500±	75%
3	44	30	1-1,000±	1-100 2	1-100+	Good	99	9	3	1-10,000±	1-500 5	1-500±	75%
4	18	10	1-5,000±	1-500 5	1-500±	Good	100		1	1-5,000±	1-500 6	1-500±	75%
5	17	21	1-5,000±	1-100 2	1-500±	Good	101	2	12	1-5,000±	1-500 5	1-500±	75%
6	26	4	1-5,000±	1-500 4	1-500±	Good	102	29	6	1-5,000±	1-500 4	1-500±	Fair
7	24	16	1-5,000±	1-100 3	1-500±	75%	103	15	30	1-10,000±	1-500 3	1-500±	50%
8	43	5	1-5,000±	1-500 3	1-500±	50%	104	30	15	1-10,000±	1-500 3	1-500±	50%
9	30	8	1-5,000±	1-500 6	1-500±	Good	105	23	1	1-1,000±	1-500 6	1-500±	75%
10	13	3	1-10,000±	1-500 4	1-500±	Good	106	29	40	1-5,000±	1-500 6	1-500±	50%
11	10	23	1-5,000±	1-100 4	1-500±	Good	107		8	1-5,000±	1-100 3	1-100±	75%
12	1	11	1-1,000±	1-500 3	1-500±	50%	108	24	1	1-5,000±	1-500 4	1-500±	None
			Season 1918				109	39	10	1-5,000±	1-500 5	1-500±	50%
13	21	12	1-10,000±	1-500 4	1-10,000±	50%	110	1	9	1-5,000±	1-500 5	1-500±	Fair
14	8	5	1-1,000±	1-100 2	1-10,000±	None	111	12	9	1-10,000±	1-500 5	1-500±	50%
15			1-1,000±	1-500 5	1-10,000±	None	112	30	5	1-10,000±	1-500 5	1-500±	50%
16		6	1-1,000±	1-500 4	1-500±	Good	113	8	27	1-5,000±	1-500 5	1-500±	75%
17	32	3	1-5,000±	1-100 3	1-500±	Fair	114	3	2	1-5,000±	1-500 5	1-500±	Fair
18	22	15	1-5,000±	1-500 4	1-500±	75%	115	30	5	1-5,000±	1-500 5	1-500±	50%
19	22	18	1-5,000±	1-500 5	1-500±	Good	116	41	17	1-5,000±	1-100 3	1-100±	75%
20	24	30	1-10,000±	1-500 5	1-10,000±	None	117	2	23	1-10,000±	1-500 6	1-500±	Fair
21	38	20	1-5,000±	1-100 1	1-500±	50%	118	8	23	1-10,000±	1-500 4	1-500±	75%
22	25	4	1-5,000±	1-100 2	1-500±	Good	119	Infant	21	1-5,000±	1-500 5	1-500±	50%
23	24	4	1-5,000±	1-500 6	1-500±	75%	120	Infant	21	1-5,000±	1-500 5	1-500±	Good
24	32	25	1-10,000±	1-500 5	1-10,000±	None	121	24	12	1-5,000±	1-500 5	1-500±	Fair
25	26	20	1-1,000±	1-100 1	1-500±	Fair	122	30	8	1-5,000±	1-500 5	1-500±	75%
26	1	35	1-5,000±	1-500 3	1-500±	75%	123	12	5	1-10,000±	1-500 5	1-500±	75%
27	28	14	1-1,000±	1-500 4	1-500±	Good	124	10	33	1-20,000±	1-500 4	1-500±	50%
28	28	26	1-5,000±	1-500 5	1-500±	Good	125	35	5	1-10,000±	1-500 3	1-500±	75%
29	24	37	1-1,000±	1-500 4	1-500±	75%	126	2	7	1-5,000±	1-500 4	1-500±	75%
30	39	17	1-5,000±	1-500 3	1-500±	Fair	127	2	47	1-5,000±	1-500 5	1-500±	Fair
31	27	4	1-1,000±	1-500 6	1-500±	Good	128	14	10	1-5,000±	1-500 5	1-500±	Fair
32	7	10	1-5,000±	1-500 4	1-500±	75%	129	17	2	1-5,000±	1-500 4	1-500±	50%
33	27	25	1-5,000±	1-500 4	1-500±	None	130	33	3	1-5,000±	1-500 3	1-500±	Fair
34	18	22	1-5,000±	1-500 6	1-500±	50%	131	20	10	1-1,000±	1-500 5	1-500±	75%
35	19	15	1-5,000±	1-500 3	1-500±	50%	132	29	10	1-1,000±	1-500 5	1-500±	50%
36a	27	10	1-1,000±	1-500 4	1-500±	Fair	133	19	15	1-5,000±	1-500 5	1-500±	50%
36	18	20	1-5,000±	1-500 4	1-500±	Good	134	16	6	1-5,000±	1-500 3	1-500±	None
36a	30	5	1-1,000±	1-500 4	1-500±	Good	135	27	12	1-10,000±	1-500 5	1-500±	50%
37	32	13	1-10,000±	1-100 3	1-500±	75%	136	49	5	1-20,000±	1-500 4	1-500±	75%
38	13	37	1-1,000±	1-100 3	1-500±	75%	137	34	1	1-20,000±	1-500 3	1-500±	50%
39	4	10	1-5,000±	1-500 3	1-500±	Good	138	26	8	1-5,000±	1-500 4	1-500±	Fair
40	19	4	1-5,000±	1-500 3	1-500±	50%	139	33	2	1-5,000±	1-500 3	1-500±	75%
41	18	19	1-5,000±	1-500 5	1-500±	75%	140	15	6	1-5,000±	1-500 5	1-500±	50%

42	24	15	Season 1919	1-10,000±	1-100 5	75% Good	142	8	2	1-5,000±	1-500 4	75%
43	39	45		1-10,000±	1-500 3	Good	143	5	35	1-5,000±	1-500 5	None
44	3	8		1-10,000±	1-100 2	50%	145	32	10	1-10,000±	1-500 5	Fair
45	32	8		1-10,000±	1-100 4	Good	146	20	6	1-1,000+	1-500 4	Fair
46	20	13		1-10,000±	1-100 5	Fair	147	10	20	1-5,000+	1-500 4	75%
47	1	38		1-5,000±	1-500 4	Good	148	35	5	1-5,000+	1-500 5	Fair
48	1	20		1-10,000±	1-100 2	50%	149	22	8	1-10,000±	1-500 4	75%
49	50	3		1-10,000±	1-500 5	Good	150	33	4	1-5,000±	1-500 5	50%
50	8	9		1-10,000±	1-100 2	Good	151	16	4	1-5,000±	1-500 3	75%
51	29	31		1-10,000±	1-100 3	Good	152	24	2	1-5,000±	1-500 4	50%
52	37	11		1-5,000±	1-500 5	75%	153	9	45	1-10,000±	1-500 5	Good
53	7	24		1-20,000±	1-100 3	Good	154	20	2	1-5,000+	1-500 4	75%
54	30	9		1-20,000±	1-100 1	None	155	24	6	1-5,000±	1-500 5	50%
55	37	4		1-10,000±	1-100 4	None	156	21	20	1-5,000±	1-500 5	75%
56	16	7		1-10,000±	1-500 4	Fair	157	30	25	1-5,000±	1-500 5	Fair
57	19	12		1-5,000+	1-100 4	50%	158	4	3	1-500+	1-500 6	50%
58	26	6		1-1,000±	1-500 5	Good	159	10	20	1-10,000±	1-500 4	50%
59	17	19		1-5,000±	1-500 3	50%	160	18	4	1-5,000±	1-100 1	75%
60	2	17		1-5,000±	1-100 4	75%	161	17	3	1-10,000±	1-500 4	75%
61	28	16		1-5,000±	1-100 4	Good	162	29	5	1-10,000±	1-500 4	Fair
62	27	22		1-10,000±	1-500 5	75%	163	36	2	1-20,000±	1-500 5	Fair
63	22	30		1-10,000±	1-500 5	75%	164	7	5	1-1,000+	1-500 5	75%
64	10	3		1-500±	1-100 2	Good	165	19	1	1-500+	1-500 4	75%
65	6	10		1-500+	1-100 4	Good	166	30	25	1-20,000±	1-500 5	75%
66	45	5		1-10,000±	1-100 3	Good	167	5	5	1-5,000±	1-500 5	50%
67	4	10		1-10,000±	1-100 2	Good	168	15	4	1-5,000±	1-100 1	75%
68	32	3		1-1,000±	1-500 4	Good	169	27	3	1-10,000+	1-100 2	75%
69	38	7		1-20,000±	1-100 2	None	170	29	1	1-1,000+	1-500 3	50%
70	22	6		1-1,000±	1-100 4	Good	171	10	8	1-10,000+	1-100 1	Good
71	30	4		1-10,000±	1-500 2	Fair	172	14	10	1-10,000±	1-100 4	75%
72	27	7		1-5,000±	1-100 4	None	173	26	2	1-10,000±	1-500 5	Good
73	16	45		1-10,000±	1-500 5	75%	174	19	2	1-5,000±	1-500 4	Fair
74	8	26		1-5,000±	1-100 4	Good	175	18	7	1-10,000±	1-500 5	75%
75	49	20		1-10,000±	1-100 1	Good	176	8	5	1-1,000±	1-100 2	50%
76	30	16		1-500+	1-100 3	Good	177	33	2	1-1,000±	1-100 3	Fair
77	36	37		1-10,000±	1-500 4	Good	178	29	5	1-5,000+	1-100 3	50%
78	17	10		1-10,000±	1-500 4	50%	179	8	9	1-10,000±	1-500 3	75%
79	25	12		1-5,000+	1-100 3	Fair	180	26	10	1-1,000+	1-100 1	50%
80	34	3		1-5,000+	1-500 5	Good	181	3	7	1-10,000±	1-100 2	75%
81	1	3		1-5,000±	1-100 2	Fair	182	30	8	1-10,000±	1-500 5	50%
82	36	4		1-5,000±	1-500 5	None	183	10	15	1-10,000±	1-500 4	75%
83	22	18		1-5,000±	1-100 1	Fair	184	35	15	1-10,000±	1-500 5	50%
84	16	4		1-10,000±	1-100 1	Fair	185	11	28	1-10,000±	1-500 4	50%
85	38	6		1-10,000±	1-100 1	Fair	187	1	28	1-10,000±	1-500 4	Good
			Season 1920	1-5,000±	1-500 5	Fair	188	16	6	1-20,000±	1-100 4	50%
86	14	7		1-10,000±	1-500 5	50%	189	20	10	1-1,000±	1-100 2	Fair
87	20	6		1-10,000±	1-500 4	50%	190	6	13	1-10,000±	1-500 5	75%
88	41	8		1-10,000±	1-500 5	50%	191	30	10	1-5,000+	1-500 5	50%
89	18	10		1-20,000±	1-500 5	75%	192	13	4	1-5,000±	1-500 6	Fair
90	15	2		1-5,000±	1-100 2	Good	193	25	5	1-5,000±	1-100 3	50%
91	28	20		1-10,000±	1-500 4	75%	194	4	5	1-5,000±	1-500 5	Fair
92	10	20		1-5,000±	1-100 4	75%	195	8	14	1-10,000±	1-500 6	50%
93	8	12		1-500+	1-100 4	Fair	196	15	20	1-10,000±	1-500 6	75%
94	33	2		1-5,000+	1-500 5	75%	197	27	25	1-10,000±	1-500 6	Fair
95	35	35		1-20,000±	1-500 5	75%	198	15	20	1-10,000±	1-500 6	75%
96	12	5		1-10,000±	1-500 4	75%	199	28	20	1-5,000±	1-500 5	Fair

* Fair in result column means practically free from symptoms, good means entirely free

did not react to a 1 500 dilution of the pollen, 8 25 per cent did react more or less to a 1 500 dilution, and 3 25 per cent reacted to a 1 1,000 dilution but no higher. Therefore, in comparison with ragweed, in only 3 25 per cent of the cases in Table 1 could golden rod pollen be assumed as a possible cause of hay-fever. From the investigations of Scheppegrell⁷ and others we know that the pollens of golden rod, sunflower, golden glow, daisy and aster are not carried by the wind, and that their pollens are heavy and shed very sparingly, so that for these reasons patients are not exposed to these pollens unless the flowers are kept in the house or unless sensitive patients smell of the flowers, naturally, the patient can refrain from such intimate contact and need not be treated for these.

Since the construction of Table 1 may not be entirely clear, the case of the second patient will be discussed in detail as an example of all of the remaining cases presented in this and similar tables. Patient 2 began to have hay-fever at the age of 8, he had had it for seven years, previous to treatment his skin test with ragweed pollen was slightly positive with a 1 5,000 dilution, therefore, treatment was begun with 0 15 c c of 1 10,000 dilution. The schedule of treatments was carried out until he was given two treatments with the 1 100 dilution, at the end of treatment his skin test was positive with the 1 100 dilution, but it should be assumed that the test was negative with weaker dilutions, namely, 1 500, etc., the result was that the patient was free from hay-fever during the August and September season in 1917. Other symbols that may need explanation are the following. In the result column good means entirely free from symptoms, fair means practically free, that is, not entirely free from symptoms but much better than 75 per cent, 75 per cent and 50 per cent mean that amount of freedom or benefit, none means no benefit or no relief, however, some of these patients did claim to be 25 per cent relieved, but I do not wish to tabulate so little benefit.

The results of treatment from the symptomatic standpoint in the 202 patients presented in Table 1 were as follows. 45 patients, or 22 + per cent, had no symptoms, 36 patients, or 17 + per cent, were practically free from symptoms, 61 patients, or 30 + per cent, were benefitted at least 75 per cent, 47 patients, or 23 + per cent, were benefitted only 50 per cent, and 13 patients, or 6 5 per cent, were not benefitted at all. Correlation of symptomatic benefit with the amount of treatment brings out the following facts. Of the forty-five patients who were entirely free from symptoms, twenty-three were given one or more treatments with the 1 100 dilution and twenty others were given four or more treatments with the 1 500 dilution. Of the thirty-six patients who

⁷ Scheppegrell, W. Hay-Fever and Hay-Fever Pollens, Arch Int Med 19.959 (July) 1917

were practically free from symptoms, one-fourth received the 1 100 dilution from one to three times, practically one-half of the series were given the 1 500 dilution five or six times, and nearly one-fourth were treated four times with this dilution. Of the sixty-one patients who were 75 per cent improved, practically one-fifth were treated from one to five times with the 1 100 dilution, nearly one-half were treated five or six times with the 1 500 dilution, a few more than one-third were treated four times with the 1 500 dilution. Of the forty-seven patients who were 50 per cent relieved, only seven, or approximately one seventh, were treated with the 1 100 dilution, approximately three eighths were given the 1 500 dilution five or six times, about one fifth were given four treatments with the 1 500 dilution, and nearly one fourth were given only three treatments with the 1 500 dilution. The thirteen patients who were not benefited will be discussed in detail later. Therefore, it is very evident that one or more treatments with the 1 100 dilution yielded the best results, and that five or more treatments with the 1 100 dilution yielded the best results, and that five treatments with the 1 500 dilution gave considerable better results than four treatments with this dilution, although four treatments with the 1 500 dilution are well worth giving, as the number of patients who were treated three times with the 1 500 dilution increased, the amount of benefit gradually decreased, until nearly one quarter of the 50 per cent benefited fell into this group, in other words, only three treatments with the 1 500 dilution give poor results in comparison to giving four or better still five treatments with this dilution. When one stops to consider that the fifth or sixth treatment with the 1 500 dilution is equivalent in amount of protein to at least one treatment with the 1 100 dilution, it is very evident that five or six treatments with the 1 500 dilution, or one treatment with the 1 100 dilution, is the ideal treatment.

Although little information is obtained by analyzing the table in regard to why some patients were more benefited than others, and why some were not benefited at all, after all it does seem worth doing.

Concerning the thirteen patients who were not improved, none gave positive skin tests with the pollens of daisy, golden rod, golden glow or sunflower. The treatment of Patient 144 was given at irregular intervals, Patient 134 lost three weeks during treatment so that he could be given only three doses of the 1 500 dilution, Patient 108 was given only four doses of the 1 500 dilution, even though many other patients were greatly benefited by similar treatment, it may be fair to assume that the negative result with these three particular cases was due to insufficient treatment. Patient 83 gave as positive a skin test at the end of treatment as he did before treatment, and the last treatment produced an anaphylactic shock manifested by urticaria.

Patients 72, 69 and 54 gave positive skin tests with a 1 1,000 dilution at the end of treatment, in spite of the fact that they all had received the 1 100 dilution one or more times. It may be safely stated that in the case of these four patients treatment did not desensitize the skin, and it is likewise probably true that, for some unknown reason, the patient's mucous membranes were not desensitized or that there was lack of union between the patients' antibodies and the treatment antigen. Patient 55 received four doses of the 1 100 dilution and at the end of treatment no dilution stronger than the 1 100 gave a positive reaction, and Patient 14 was treated with the 1 100 dilution two times so that these two patients ought to have had sufficient pollen treatment. Patients 33, 24, 21 and 15 all received the 1 500 dilution five times so that they also did not fall short of the average amount of treatment. The varying ages of onset and years of duration of symptoms, and the approximately equal distribution between the two sexes and between the three different years, furnish no clues toward the cause of failure from treatment. It would seem that we must blame the individuality or idiosyncrasy of these particular patients for the time being, and, therefore, it should be admitted that with our present knowledge there are a few hay-fever victims who are not benefited by preseasonal pollen therapy.

Of the forty-seven patients who were only 50 per cent improved, three showed evidence of lack of desensitization, as was noted in the previous paragraph, in that one case following four treatments with the 1 500 dilution and another following six similar treatments gave positive skin tests with a 1 1 000 dilution of the pollen protein, the third gave a positive skin test with the 1 5,000 dilution following two doses of 1 100, and it is interesting that this patient was not shocked. It is difficult to explain why fifteen patients who were treated five times with the 1 500 dilution, and three patients who were similarly treated six times should not receive more than 50 per cent relief when so many more experienced greater benefit from the same treatment. It is still more difficult to understand why the six patients who were treated from one to four times with the 1 100 dilution of pollen protein should be only 50 per cent improved, especially since at the end of treatment one patient gave negative tests with the whole pollen protein and two others gave positive reactions with no stronger than the 1 100 dilution.

Attention should be called to the decreased sensitivity of the patient as evidenced by the skin test following a satisfactory series of treatments. Fifty-one patients were tested at the end of treatment. One third of these gave a more or less positive skin test with the 1 100 dilution but did not react to a more dilute solution of the pollen protein, the same number gave a more or less positive skin test with the 1 500 dilution but failed to react to a more dilute pollen protein solution, one half of these were given treatments with the 1 100 dilution, and the

other half with the 1 500 dilution, a few were given only three doses of the 1 500 dilution, five failed to react at all with the 1 100 dilution, four were treated with the 1 100 dilution, one patient who was treated with the 1 100 dilution failed to react to the whole pollen, eight gave a more or less positive skin test with the 1 1,000 dilution, four having been treated with the 1 100 dilution without producing ill symptoms, two patients reacted with the 1 5,000 dilution of pollen protein—one, Patient 83 was shocked (urticaria) following the fifth treatment with a 1 500 dilution and was unimproved, whereas the other, Patient 178, had received two doses of the 1 100 dilution but was not shocked and was 50 per cent improved. Therefore, in all but two cases, treatment decreased the sensitivity of the patient, as evidenced by the skin test, or to be more specific, in practically three-fifths of the cases the intensity of the skin test diminished 100 times and in practically the remaining two fifths it was decreased at least twenty times, the larger the number of treatments given, the greater was the decrease in the intensity of the skin test.

Pollens, other than ragweed, that might complicate the cause and treatment of hay-fever at this season of the year in New England have already been mentioned, and to a certain extent these have been eliminated. Since, however, a few patients listed in Table 1 did react rather strongly to other pollens, it may be advisable to mention them. Patients 4, 6, 23 and 29 gave more or less positive skin tests with a 1 1,000 dilution of daisy but the first two were free from symptoms and the other two were 75 per cent benefited by six and four treatments, respectively, of a 1 500 dilution of ragweed pollen alone. Patient 26 reacted to a 1 1,000 dilution of corn pollen but was 75 per cent improved by three doses of 1 500 dilution of ragweed pollen. Patients 19, 28 and 74 reacted more or less to a 1 1,000 dilution of golden rod pollen, but following five treatments with 1 500 ragweed pollen, Patients 19 and 28 were free from symptoms, and Patient 74 was 75 per cent benefited. Therefore, for reasons already stated, it would seem that pollens other than ragweed played no part in the cause of hay-fever at this particular season.

The age at which hay-fever began, and the number of years that the patient has had hay-fever, seem to play no part in the cause or the treatment of this type of hay-fever. Neither does the sex of the patient have any bearing even on the frequency of the condition. The patients in Table 1 were equally divided between the two sexes. A number of the patients were treated at five day intervals rather than, as was usually the case, at seven day intervals. Although statistics would not reveal any information on this point, it is my impression that the seven day interval is preferable to the five day interval. The individuality of the

patient certainly plays a great part in the treatment and in the results of treatment of hay-fever, however, any definite information in regard to this point cannot be obtained

The same season in different years varies greatly in regard to the abundance of pollen, the beginning and the end of pollination and the frequency of colds that may be mistaken for hay-fever and thereby complicating the results of treatment. For example, in 1918, ragweed began to pollinate about August 8, in 1918 and 1919, frosts sufficiently heavy to stop pollination occurred about the middle of September, and even during all of September the weather was cold and rainy, so that not only was pollination below normal but head colds were frequent and the influenza epidemic was present. The season of 1920 was very favorable for ragweed, no frosts severe enough to injure it occurred and pollination continued from about August 13 until October 1, at which time the plant naturally completed pollination and went to seed. There was no epidemic of colds and very little rain. For these reasons the results of preseasonal or preventive treatment with ragweed pollen in 1920 are of great value and are a true test for the treatment. Before considering these results, however, it should be noted that the amount of treatment given in this particular year was, in general, less than that given in preceding years. On referring to Table 1, season of 1920, the following are seen to have been the results of ragweed treatment, of the 115 patients treated, 27 per cent experienced little or no hay-fever, 40 per cent were 75 per cent relieved, about 66 per cent were therefore, either 75 per cent relieved or more so, and less than 33 per cent had as little as 50 per cent relief, whereas only three patients, or 2.6 per cent, were not benefited by treatment.

Before Table 2 is discussed, attention should be called to the plants that pollinate during June and July, the early hay-fever or so-called rose cold season. In the New England States some of the compositae, the most important of which is daisy, pollinate at this time but for reasons already given daisy pollen rarely, if ever, is a cause of hay-fever. Such plants as the lilies, sorrel, buttercup and others pollinate at this time, but when tested no patient has been found to be sensitive to these, and if they ever cause hay-fever it is because of immediate nasal contact with the blossom. The rose which pollinates at this time is rarely a cause of hay-fever since it is not a wind pollinated plant. Since, however, rose is commonly suspected to be the cause of hay-fever or rose colds, and since the pollen of rose is occasionally the actual cause, skin tests with rose pollen frequently have to be done, if for no other reason than to satisfy the patient. Although in Table 2 only two patients (219 and 226) are represented as having been tested with rose pollen, thirty-three others of the fifty-two were tested with rose, and thirty-one failed to react more than doubtfully with the whole pollen of either

the red or the white rose, and the two who did give a positive skin test with the whole pollen failed to react at all with a 1 100 dilution of the pollen. The thirty-five patients who were tested with rose pollen were tested with it because they thought roses caused their hay-fever, and the other patients in Table 2 were not tested with it because they were sure that roses did not cause their hay-fever. Therefore, it would seem that in New England roses were rarely the chief cause of hay-fever.

The cause of hay-fever during June and July in the New England States is practically limited to the pollens of the grass family. Lawn grass begins to pollinate early in May, as a rule, but since it is rare for hay-fever to begin early in May, lawn grass pollen is probably rarely, if ever, the chief cause of hay-fever. Since, however, lawn grass continues to pollinate throughout the summer even when it is repeatedly mowed closely, with those patients who are sensitive to the pollens of other grasses and are either not treated or are insufficiently treated with the particular grass pollen that is the chief cause of hay-fever, exposure to lawn grass pollen may be a complicating cause or may aggravate the symptoms of hay-fever. Orchard grass pollinates during July, but this type of grass grows in more or less secluded places, and it is not commonly encountered. The same thirty-five patients who were tested with rose pollen were also tested with orchard grass pollen. Thirty of them failed to give positive skin tests with the whole pollen, and the five who gave a positive test with the whole pollen failed to react with a 1 100 dilution of the pollen extract, therefore, orchard grass rarely, if ever, is an actual cause of hay-fever. Since corn is a member of the grass family, and since the table variety pollinates during July, it must be considered among the possible causes of early hay-fever. Forty of the fifty-two patients presented in Table 2 were tested with sweet corn pollen, twenty-two failed to react at all, and eighteen gave a positive skin test with the whole pollen, although no tests with a 1 100 dilution of the pollen extract were positive. The large number of positive tests with the whole pollen was probably due, in part, at least, to the very large amount of protein present in corn pollen in comparison to the small amount of protein present in other pollens. Corn pollen is very heavy, it rapidly descends to the ground and is carried by air currents or wind only a few feet, therefore, intimate exposure would be required to produce symptoms, furthermore, only rarely would a person be unable to avoid it. Therefore, corn pollen must rarely, if ever, be considered a cause of hay-fever in the New England States where no large acreage exists in any locality as is the case in the West. This same statement is, likewise, true of wheat, oats, barley and rye.

The grasses, then, with which we are concerned in New England are June grass, timothy and redtop, the pollens of which are light and are carried by wind considerable distances.⁷ June grass begins to pollinate

TABLE 2—PRESEASONAL TESTS AND TREATMENTS WITH GRASS POLLENS

Patient	Age of Onset	Duration	Tests Before Treatment			Number of Treatments With Final Dilution			Tests at End of Treatment			Result of Treatment
			Tests Before Treatment		June Grass	Number of Treatments With Final Dilution		Tests at End of Treatment				
			Timothy	Red Top		Timothy	Red Top	Timothy	Red Top	June Grass		
201	50	10	1-1,000±	1-1,000±		1-100 2	Season 1917					Good
202	14	8	1 10,000±	1-1,000±		1-500 3						Good
203	23	18	1-500±	1-1,000±			1-100 3					Good
204	12	40	1-5,000±	1-1,000±		1-500 3						Good
205	30	5	1-1,000±	1-1,000±		1-100 1						75%
206	10	9	1-1,000±	1-100±		1-500 3			1-100 0			Good
207	27	10	1-5,000±	1-500±		1-100 3			1 100 0			Good
208	6	5	1-1,000±	1-100±		1-100 3			1-100±			Good
209	7	14	1-5,000±	1-5,000±								Good
210	25	7	1-10,000±	1-5,000±		1-100 1	Season 1918					Good
211	34	10	1-1,000±	1-5,000±		1-100 1	1-100 1					Good
212	27	18	1-1,000±	1-1,000±		1-100 2	1-100 2					Good
213	19	4	1-5,000±	1-1,000±		1-100 1						Good
214	32	13	1-500±	1-500±		1-100 2						Good
215	13	6	1-5,000±	1-500±		1-100 3						Fair
216	5	11	1-1,000±	1-100 0		1-100 4						Good
217	20	15	1-5,000±	1-1,000±	1-1,000±	1-100 2	Season 1919		1-100±	1-1,000±	1-1,000±	Good
218	33	12	1-1,000±	1-1,000±	1-1,000±	1-100 3			1-100±	1-500±	1-500±	Good
220	7	2	1-5,000±	1-1,000±	1-5,000±	1-100 3			1-100±	1-500±	1-500±	Good

221	20	8	1-1,000+	1-1,000+	1-1,000+	1-500 3	1-500 4	Season 1920	1-500 4	1-500±	1-500±	1-500±	50% Good 50% Fair
222	1	37	1-5,000±	1-1,000±	1-1,000±	1-500 4	1-500 4						
223	8	3	1-5,000±	1-5,000±	1-5,000±	1-500 5							
224	12	5	1-1,000±	1-1,000±	1-1,000±	1-500 4							
225			1-1,000±	1-500+	1-500±								
227	9	2	1-5,000±	1-5,000±	1-5,000±	1-500 4	1-500 4						75% 75% 50% 75% 50% 50% 75% 50% Fair 50% None 50% Good 75% None Fair 75% Fair Fair None 75% 75% Fair Good Fair Good
228	3	11	1-1,000±	1-1,000±	1-1,000±	1-500 3	1-500 3						
229	23	4	1-5,000±	1-1,000±	1-1,000±	1-500 6	1-500 4						
230	23	6	1-5,000±	1-5,000±	1-5,000±	1-500 4	1-500 2						
231	8	5	1-10,000±	1-10,000±	1-10,000±	1-500 4	1-500 4						
232	29	15	1-1,000±	1-1,000±	1-1,000±	1-500 5	1-500 4						
233	11	3	1-10,000+	1-10,000+	1-10,000+	1-500 3	1-500 3						
234	23	25	1-1,000±	1-1,000±	1-1,000±	1-500 3	1-500 3						
235	21	30	1-10,000±	1-1,000±	1-1,000±	1-500 4	1-500 1						
236	36	11	1-5,000±	1-1,000±	1-1,000±	1-500 3	1-1,000 2						
237	13	4	1-1,000+	1-1,000+	1-1,000+	1-500 4							
238	12	4	1-5,000±	1-5,000±	1-5,000±	1-500 4	1-500 2						
239	20	2	1-500+	1-5,000±	1-5,000±	1-500 4	1-500 4						
240	22	8	1-5,000±	1-5,000±	1-5,000±	1-100 1	1-500 4						
241	15	12	1-1,000+	1-5,000±	1-5,000±	1-100 1	1-500 2						
242	7	5	1-500+	1-500±	1-500±	1-500 4	1-500 4						
243	6	40	1-5,000+	1-5,000±	1-5,000±	1-500 2	1-500 5						
244	30	22	1-5,000+	1-5,000+	1-5,000+	1-500 4	1-500 4						
245	19	1	1-1,000±	1-500+	1-1,000±	1-500 5	1-500 5						
246	1	20	Negative	1-1,000±	Negative	1-100 4	1-100 4						
247	4	4	1-5,000+	1-1,000±	1-5,000+	1-500 5	1-500 5						
248	1	1	1-5,000+	1-500±	1-1,000+	1-500 5	1-500 5						
249	21	20	1-5,000±	1-1,000+	1-1,000+	1-500 5	1-500 5						
250	16	19	1-5,000±	1-1,000+	1-5,000+	1-500 5	1-500 5						
251			1-500±	1-100 0	1-500±	1-500 5	1-500 5						
252	14	9	1-500±	1-500±	1-500±	1-100 5	1-500 5						
253	3	3	1-1,000±	1-1,000±	1-1,000±	1-100 5	1-500 5						
254			Rose 1-500+	Rose 1-500+	Rose 1-500+								
255	26	38	Negative	Negative	Negative	1-500 5	1-500 5						
256						Negative	Negative						

some years as early as the middle of May, when the season is very late, it does not pollinate before the last day or two in May, pollination continues for about three weeks. Timothy and redtop begin to pollinate between the middle of June and the first of July, depending on the season, and pollination continues until the middle or last of July, usually, the season of pollination lasts about six weeks. Therefore, in order to complete the schedule of treatments mentioned earlier in this paper as being desirable, preseasonal treatment with June grass must begin the first of March at the latest, and with timothy and redtop it must begin the middle of March.

Analysis of Table 2 shows the results of preseasonal treatment for the early type of hay-fever. Of the fifty-two patients treated, twenty-one, or 40.4 per cent, were free from symptoms, nine, or 17.3 per cent, were practically free from symptoms, ten, or 19.2 per cent, were 75 per cent relieved, nine, or 17.3 per cent, were 50 per cent relieved, and three, or 5.8 per cent, were not benefited. Twenty-eight of the patients were treated with timothy grass pollen alone, three with redtop alone, one with rose alone, two with timothy and redtop together, one with redtop and rose together and seventeen with timothy and June grass pollens together. Although these facts are of interest, much valuable information is missed unless particular attention is given to each year, therefore, each year will be discussed separately.

During the season 1917, nine patients were treated. Only one patient (203) was treated with redtop pollen, because this patient was more sensitive to dilutions of redtop pollen extract than to timothy pollen extract; the remaining eight patients were treated with timothy pollen extract alone because all but two were more sensitive to timothy pollen extract than to redtop pollen extract and since these two were equally sensitive to both pollen extracts, timothy was selected for treatment because it was thought that timothy was more prevalent than redtop. Judging from the excellent results and the decreased sensitivity of the patients, there is every reason to believe that all patients had a sufficient number of treatments, that all but two, who were 50 and 75 per cent, respectively, relieved by treatment with redtop pollen extract, were treated by the proper pollen. As a matter of fact, the two patients who were only 50 per cent and 75 per cent relieved had most of their symptoms during June rather than in July which fact would make one suspicious that some pollen other than those tested was the cause of symptoms, tests were made with orchard grass, corn and rose, none of which reacted in a dilution of 1:100, but June grass, which also pollinates during June, was not tested. With the exception of these two cases, timothy pollen extract would seem to have protected against redtop exposure since five of the patients were very sensitive to redtop pollen extract.

During 1918, seven patients were treated, and all but one were free from symptoms and that one was practically free. All patients were treated one or more times with the 1:100 dilution and all but two were treated with timothy pollen extract alone, these two were treated with the extracts of timothy and redtop pollens together. Therefore, the same conclusions that were drawn from the 1917 series hold true for the 1918 series, namely, that all patients had a sufficient number of treatments, that treatment with timothy pollen extract protects against redtop exposure, that, as a rule, patients are more sensitive to timothy pollen than to any other pollen prevalent at that time, with the possible exception of June grass which was not tested, and that the reason why two patients were treated with redtop pollen in addition to timothy pollen was because one was more sensitive to redtop than to timothy and the other was equally sensitive to both.

During 1919, eight patients were treated with the result that five were free from symptoms, one other was practically free, and two were only 50 per cent relieved. Of the two patients (221 and 224) who were only 50 per cent benefited, one had only three treatments with timothy pollen extract 1:500 and the other was treated with redtop instead of timothy, both patients had more symptoms in June than in July. Therefore, as regards timothy and redtop the same conclusions that were true of 1917 and 1918 were equally true for 1919. In 1919, tests were made with June grass and on referring to the table, season 1919, it will be seen that the eight patients reacted as strongly to June grass pollen as to redtop, some reacted more strongly to it, and all but two were as sensitive to June grass as to timothy pollen extract. It is also noted that four patients, who were tested with the three grass pollens at the end of timothy pollen treatment, showed a much greater decrease in sensitiveness to timothy pollen than to redtop or to June grass pollen. Therefore, since there is evidence that timothy pollen treatment seems to protect against redtop pollen exposure, as has already been shown, and since the patients in 1917 and 1919 who were least benefited by treatment, either by timothy or redtop, had most of their symptoms during the usual time of June grass pollination rather than during the time that timothy and redtop pollinate, and since in 1919 it was shown that early hay-fever patients are very sensitive to June grass pollen extract, it was deemed best to treat suitable patients with June grass pollen during 1920.

During the 1920 season, only three of twenty-eight patients were to any extent less sensitive to June grass pollen extract than to timothy pollen extract, and three of the patients were more sensitive to June grass pollen than to timothy. Therefore, in seventeen patients treatment was given with June grass pollen extract together with timothy pollen extract. The results of this mixed treatment were one patient

was free from symptoms, five were practically free, five were 75 per cent relieved, five were 50 per cent relieved, and one was not benefited, a very poor showing as compared to treatment with timothy pollen extract alone during the three previous years. It is also noted that only two patients who were treated with the mixture were given as many as one treatment with timothy, 1 100, which is desirable and which was the case in previous seasons when excellent results were obtained, treatment with a mixture of the two pollens did not and will not permit of sufficient treatment with either one, and in this instance it is evident that a part, at least, of the poor results, if the three former seasons are a guide, was due to insufficient treatment with timothy pollen. The absence of treatment with redtop pollen cannot explain the poor results, since in the three former seasons excellent results were obtained in the absence of redtop treatment.

Special attention is called to Patients 246, 219 and 226 because their cases are of considerable interest. Patient 246 was sensitive to redtop pollen alone and was practically free from symptoms following four treatments with the 1 100 dilution of redtop pollen extract. Not only was this patient free from symptoms while in New England, but he was also free while in Wyoming and was practically free while in California. Patient 219, who was practically equally sensitive to the pollens of the three grasses and to rose, was practically free from symptoms following treatment with equal parts of redtop and rose pollen extracts. Patient 226, who was sensitive to rose pollen only, was free from symptoms following treatment with rose pollen extract. Therefore only two rose cases were encountered in the four seasons during which fifty grass cases were tested and treated.

My conclusions, based on a four year's experience with the treatment of June and July hay-fever, are. While the pollen of timothy grass is the chief cause of hay-fever, and sufficient treatment with it alone gives excellent results, it is necessary to test all patients with the pollens of redtop, June grass and rose since an occasional patient is more sensitive to one of these pollens than to timothy pollen, and, therefore, such a patient may need treatment with one of them rather than with timothy pollen. When the same patient, as is frequently the case, is equally sensitive to the dilutions of the pollen extracts of timothy, redtop and June grass, it is advisable to treat the patient with timothy pollen extract alone because there is sufficient evidence that treatment with timothy pollen will protect against redtop pollen exposure, provided one or more treatments are given with the 1 100 dilution of the pollen extract, timothy and redtop pollinate at the same time, and redtop is less prevalent than timothy. Sufficient treatment with timothy pollen extract seems to protect also against June grass pollen exposure, but since, as is often the case, June grass pollination is well

advanced or even sometimes completed before timothy pollination begins, the patients being treated with timothy have not had sufficient treatment with timothy pollen to protect them completely against June grass pollen, consequently some of these patients will have more or less symptoms from June grass exposure, as happened in 1917 with Patients 203 and 205, in 1919, with Patients 221 and 224, and in 1920 with Patients 232 and 249. In 1918, when June grass pollination was

TABLE 3—INSUFFICIENT POLLEN TREATMENT

Patient	Age of Onset	Duration	Tests Before Treatment	Number of Treatments With Final Dilution	Result of Treatment
Ragweed					
253	32	13	1-10,000+	1-1,000 2	None
254	22	4	1-5,000±	1-1,000 3	50%
255	3	10	1-5,000±	1-1,000 3	75%
256	18	5	1-1,000±	1-1,000 3	50%
257	14	24	1-5,000±	1-1,000 2	50%
258	16	45	1-5,000±	1-1,000 2	50%
259	19	15	1-5,000±	1-1,000 2	25%
260	28	14	1-1,000+	1-1,000 2	75%
261	13	9	1-5,000±	1-1,000 3	50%
262	39	10	1-5,000+	1-1,000 3	None
263	31	2	1-1,000±	1-500 2	None
264	3	9	1-10,000+	1-5,000 4	None
265	6	12	1-10,000+	1-1,000 2	50%
266	16	7	1-10,000±	1-1,000 3	75%
267	44	10	1-10,000±	1-5,000 5	50%
268	16	45	1-10,000±	1-500 2	50%
269	27	12	1-10,000±	1-500 2	50%
269	44	6	1-5,000+	1-500 2	50%
271	14	9	1-5,000±	1-1,000 2	None
272	9	2	1-5,000±	1-5,000 5	50%
273	14	10	1-5,000+	1-500 2	None
274	29	3	1-10,000±	1-5,000 1	50%
275	3	49	1-10,000±	1-500 2	None
276	3	6	1-20,000±	1-5,000 3	50%
277	9	9	1-10,000±	1-500 2	50%
278	3	20	1-10,000±	1-5,000 4	50%
279	16	16	1-10,000±	1-500 1	None
280	5	19	1-10,000±	1-1,000 2	50%
281	18	3	1-10,000±	1-500 2	75%
282	35	12	1-10,000±	1-1,000 2	Fair
283	31	6	1-10,000±	1-500 2	75%
284	35	15	1-10,000±	1-500 2	50%
285	13	16	1-1,000+	1-500 2	75%
294	11	7	1-5,000±	1-500 2	50%
295	31	5	1-5,000±	1-500 2	None
296	20	17	1-5,000±	1-500 1	50%
297	22	25	1-5,000±	1-500 2	75%
298	11	7	1-5,000±	1-500 2	50%
299	37	18	1-5,000±	1-500 2	50%
270	41	7	1-5,000±	1-500 2	50%
Timothy					
286	30	4	1-5,000±	1-1,000 2	None
287	19	10	1-10,000±	1-5,000 3	None
288	30	5	1-5,000±	1-1,000 3	50%
290	14	6	1-5,000±	1-1,000 2	None
291	8	3	1-1,000±	1-500 2	50%
292	14	14	1-10,000±	1-500 2	None
293	22	5	1-10,000±	1-5,000 5	50%

late, timothy treatment was successful, and the few cases in Table 2 that were tested with the three grass pollens at the end of much timothy treatment alone, were considerably less sensitive to all of the grass pollens and all of the patients were free from symptoms. Unless treatment with June grass pollen is begun earlier than is customary with timothy pollen, patients will not be treated sufficiently with June

grass pollen Therefore, treatment with both timothy pollen and June grass pollen extracts together will not only be insufficient to protect against early June grass pollination (Patients 229, 233, 234 and 238, all of whom had symptoms in late May and early June), but the combination of the two pollen extracts necessarily retards and diminishes the amount of treatment that might be given with timothy alone Table 2 shows this to be the usual case Therefore, I am opposed to preseasonal treatment with pollen mixtures, except on rare occasions, and in the case of June grass it is better to take a chance on a late season with timothy protection or even an early season with few symptoms than to treat with it in conjunction with timothy when timothy treatment is essential

In Table 3 are presented patients who had insufficient pollen treatment, and by insufficient is meant that for some reason or other the complete schedule of treatments, as outlined early in this paper, could not be given A few of the patients did receive the 1 500 dilution of the pollen extract two times, but for reasons already stated I consider this insufficient treatment

In Table 3 are presented forty patients who were given insufficient treatment with ragweed pollen extract, and seven patients similarly treated with timothy pollen extract The possibility of sensitization to pollens, that might be the cause of symptoms, other than ragweed and timothy will not be considered in the light of previous discussion The results were as follows Of the forty-seven patients in Table 3, none were free from symptoms, one was practically free, seven were 75 per cent benefited, twenty-five were 50 per cent benefited, one was 25 per cent benefited and the remaining thirteen were not benefited Therefore, although a few patients may be from 50 to 75 per cent benefited by as small a number as two treatments of the 1 500 dilution, these results are offset by the number of failures resultant from similar treatment, and this is still more evident when a smaller amount of treatment than two doses of the 1 500 dilution is given The results shown in Table 3 verify what has already been proven in Tables 1 and 2, namely, that giving less than three preseasonal treatments with the 1 500 dilution of pollen extract is undesirable

In Table 4 are presented seventy-three patients who were treated with ragweed pollen extract two years in succession Six patients were treated in the two seasons 1917 and 1918, fifteen, in 1918 and 1919, and fifty-two in 1919 and 1920

Analysis of Table 4 shows that for the first year of treatment, of the seventy-three patients, twenty-eight, or 38 + per cent, were free from symptoms, sixteen, or 22 per cent, were practically free, fourteen, or 19 + per cent, were 75 per cent relieved, twelve, or 16 4 per cent, were 50 per cent benefited, and three, or 4 + per cent, were

TABLE 4—PRESEASONAL TESTS AND TREATMENT WITH RAGWEED
POLLEN TWO SUCCESSIVE YEARS

Patient	Age of On set	Duration	Season 1917				Season 1918			
			Tests Before Treatment	No Treatments With Final Dilution	Tests at End of Treatment	Result of Treatment	Tests Before Treatment	No Treatments With Final Dilution	Tests at End of Treatment	Result of Treatment
300	16	10	1-1,000±	1-100 2		Good	1-1,000±	1-100 2		Good
301		10	1-1,000±	1-100 3		Good	1-5,000±	1-100 1	1-500±	Good
302	45	3	1-5,000±	1-100 1		Good	1-500±	1-500 5		Good
303	39	10	1-5,000±	1-500 4		Good	1-5,000±	1-100 2		Good
304	20	10	1-5,000±	1-100 3		Good	1-5,000±	1-500 5		75%
305	32	6	1-5,000±	1-500 4		75%	1-1,000±	1-100 1		Good
			Season 1918				Season 1919			
306	24	25	1-5,000±	1-500 5		Good	1-5,000±	1-500 5		75%
307	14	15	1-5,000±	1-100 2		Good	1-5,000±	1-100 4	1-100+	Good
308	42	8	1-5,000±	1-500 5		50%	1-5,000±	1-100 3	1-100+	75%
309	37	9	1-1,000±	1-500 8		Good	1-5,000±	1-100 3	1-100±	Good
310	3	10	1-5,000±	1-500 3		Good	1-5,000±	1-500 4		Good
311	20	25	1-5,000±	1-100 4		None	1-5,000±	1-100 1		None
312	6	26	1 5,000+	1-100 1		Good	1-5,000±	1-100 5	1-500	Good
314	3	11	1-5,000±	1-500 7		75%	1-10,000±	1-500 4		None
315	13	3	1-5,000±	1-500 4		50%	1-10,000±	1-500 3		Fair
316	34	9	1-5,000±	1-500 7		75%	1-5,000±	1-100 2		None
317	37	4	1-1,000±	1-500 2		50%	1-1,000±	1-500 4	1-100±	75%
318	12	2	1-5,000±	1-500 2		50%	1-5,000±	1-100 4	1-100±	75%
319	13	16	1-1,000±	1-500 5		Fair	1-1,000±	1-500 2		75%
320	29	6	1-5,000±	1-100 4		None	1-10,000±	1-100 3		50%
321	23	4	1-1,000±	1-500 6		75%	1-5,000±	1-500 4	1-100 0	75%
			Season 1919				Season 1920			
322	32	10	1-5,000+	1-100 4	1-100±	Good	1-5,000±	1-500 3		75%
323	39	5	1-10,000±	1-100 4	1-100+	Fair	1-5,000±	1-100 1	1-500±	75%
324	29	4	1-5,000+	1-100 4	1-100+	Good	1-5,000±	1-500 5		75%
325			1-1,000±	1-100 4	1-100+	Fair	1-1,000±	1-500 3		75%
326	50	2	1-5,000±	1-100 5	1-500±	Good	1-1,000±	1-100 2	1-500±	75%
327			1-10,000±	1-100 5	1-500±	Good	1-10,000±	1-500 5		Good
328	18	3	1-5,000±	1-500 5	1-100±	Good	1-5,000±	1-100 2		75%
329			1-5,000±	1-500 5		Good	1-5,000±	1-100 3		Fair
330	34	10	1-5,000±	1-100 5	1-100 0	None	1-1,000±	1-100 3		75%
331	14	7	1-10,000±	1-500 5		50%	1-10,000±	1-100 3		75%
332	12	9	1-5,000±	1-500 5		Fair	1-1,000±	1-500 5		75%
333	4	12	1-10,000±	1-500 5		75%	1-10,000±	1-500 5		Fair
334	10	7	1-10,000±	1-100 2	1-500±	Fair	1-10,000±	1-500 4		Fair
335	19	12	1-5,000±	1-500 4		75%	1-5,000±	1-100 1	1-5,000±	50%
336	32	12	1-10,000±	1-100 3	1-500±	75%	1-10,000±	1-500 4		50%
337	17	25	1-20,000±	1-100 2		Good	1-10,000±	1-100 1	1-500±	Fair
338	36		1-10,000±	1-500 6		Good	1-5,000±	1-500 5		Fair
339	32	10	1-10,000±	1-100 4	1-100+	Fair	1-5,000±	1-100 3		75%
340	25		1-10,000±	1-500 4		Fair	1-5,000±	1-500 5		75%
341	30	15	1-5,000±	1-500 6	1-100 0	75%	1-5,000±	1-500 2		50%
342	30	4	1-10,000±	1-100 2	1-500+	Fair	1-10,000±	1-500 4		50%
343	32	6	1-20,000±	1-100 3	1-500±	Fair	1-10,000±	1-500 4		Fair
344	5	2	1-10,000±	1-100 2		Good	1-1,000±	1-100 2		Good
345	4	34	1-10,000±	1-100 2	1-500±	75%	1-5,000±	1-100 2	1-500±	Fair
346	6	4	1-10,000±	1-500 4	1-500±	75%	1-5,000±	1-500 6	1-500 0	75%
347	14	5	1-5,000±	1-500 6	1-500+	Fair	1-5,000±	1-500 5	1-500+	50%
348	16	45	1-10,000±	1-500 2	1-1,000+	50%	1-10,000±	1-500 5	1-1,000+	Fair
349	28	10	1-10,000±	1-500 6		Good	1-5,000±	1-500 6		Fair
350	27	6	1-10,000±	1-100 4	1-100+	Fair	1-5,000±	1-500 5		50%
351	5	19	1-10,000±	1-500 3		75%	1-10,000±	1-1000 1		Fair
352	18	5	1-10,000±	1-500 4	1-500±	75%	1-5,000±	1-500 4		75%
353	27	7	1-20,000±	1-100 3	1-500±	Fair	1-10,000±	1-500 3		75%
354	38	4	1-5,000±	1-100 4	1-500±	Fair	1-5,000±	1-100 1	1-500+	Fair
355	34	12	1-10,000±	1-500 5		75%	1-5,000±	1-500 5		75%
356	28	6	1-20,000±	1-500 4		Good	1-5,000±	1-500 5		Fair
357	5	36	1-10,000±	1-100 1	1-500±	50%	1-5,000±	1-500 5		Fair
358	28	13	1-10,000±	1-500 5	1-500±	50%	1-20,000±	1-700 4		50%
359		28	1-20,000±	1-500 1		50%	1-20,000±	1-500 5	1-1,000±	75%
360	23	39	1-20,000±	1-100 5	1-500±	Fair	1-10,000±	1-100 3		Fair
361	45	7	1-1,000±	1-100 1	1-100+	Good	1-5,000±	1-500 4	1-500 0	75%
362	29	9	1-5,000±	1-500 5	1-500+	50%	1-10,000±	1-500 5		75%
363	18	10	1-10,000±	1-500 4	1-500±	Good	1-5,000±	1-500 5		Fair
364	27	20	1-10,000±	1-500 4		75%	1-5,000±	1-500 5		75%
365	31	28	1-5,000±	1-500 3		Fair	1-5,000±	1-500 3		50%
366	25	3	1-10,000±	1-500 4	1-500+	50%	1-10,000±	1-500 6	1-500+	75%
367	33	5	1-1,000±	1-100 4	1-100 0	Good	1-500±	1-500 4		75%
313	1	3	1-5,000±	1-500 5		Good	1-5,000±	1-100 2		Good
368	40	18	1-10,000±	1-100 1	1-100±	Good	1-5,000±	1-500 5		75%
369	11	8	1-5,000±	1-500 5	1-500±	Fair	1-5,000±	1-500 5		75%
370	14	18	1-5,000±	1-100 4	1-100±	Good	1-500±	1-100 2	1-100±	75%
371	32	31	1-10,000±	1-100 4	1-100+	Good	1-5,000±	1-100 2	1-500±	75%
372	7	6	1-10,000±	1-100 1	1-100±	50%	1-5,000±	1-500 6		75%

not benefited For the second year of treatment, the results were Of the seventy-three patients, twelve, or 16.4 per cent, were free from symptoms, sixteen, or 22 per cent, were practically free, thirty-three, or 45 per cent, were 75 per cent relieved, nine, or 12 per cent, were 50 per cent relieved, and three, or 4 + per cent, were not benefited On comparing the results obtained from the first year's treatment with those obtained from the second year's treatment, it is noted that the same number of patients were practically free from symptoms both years, and that the number who were 50 per cent benefited were about the same for the year, but that there were 57 per cent more all right the first year than the second year, and 57 per cent more that were 75 per cent benefited the second year than the first

Although the above results obtained by the first and second years' treatment do not differ markedly, yet this difference shows rather poorer results from the second year's treatment than from the first year's treatment, the opposite of what one would expect and desire By comparing the amount of treatment that was given the first year with that given the second year it is evident that a diminished amount of treatment during the second year would seem to explain the poorer results obtained during this year, and it may be stated, that, as a rule, it is desirable to give as much treatment with the final dilutions the second year, provided sufficient treatment is given the first year, and sometimes less treatment is required

Attention should be called to the few patients in Table 4 who were not benefited by treatment Patient 311 was treated four times with the 1:100 dilution of pollen extract the first year, and once with the same dilution the second year, and yet there was no benefit from treatment either year, although this patient was much worse during August and September, he did have symptoms throughout the year so that it is probable that there was some cause of hay-fever besides ragweed pollen It is difficult to understand why Patient 314 should be 75 per cent benefited following seven treatments with a 1:500 dilution of pollen extract the first year and not benefited by four similar treatments the second year unless the failure was due to insufficient treatment the second year There is no evident reason why Patient 316, who was similarly treated both years, was 75 per cent benefited the first year and not benefited the second year, neither is there an evident explanation why Patient 320, who was similarly treated both years, was not benefited the first year even though at the end of treatment the skin test was negative with a 1:100 dilution of the pollen, but was 75 per cent benefited the second year It happens that all of these failures occurred during the two years 1918 and 1919, and both ragweed seasons, as already described, were very similar None of these patients were sensitive to pollens other than ragweed

Fifty-seven skin tests were done at the end of treatment. In one case (335) the test was the same at the end of treatment as before treatment, and the patient was only 50 per cent benefited, a similar instance was noted in Table 1. Patient 348 gave a positive skin test with a 1:1,000 dilution of the pollen extract both years at the end of treatment, but was 50 per cent benefited the first year and was practically free from symptoms the second year. In the remaining fifty-four tests, there was the usual diminished intensity of the reaction, in one third of the cases, or those who had been treated with the 1:100 dilution of pollen extract, the skin test was positive in no dilution higher than 1:100, and in some cases even this dilution was negative, in the remaining two-thirds of the instances no dilution higher than 1:500 gave a positive skin test at the end of treatment. The sensitivity of the patients was decreased from ten to one hundred times with an average decrease of from twenty to fifty times.

The conclusions resulting from Table 4 substantiate all of those already mentioned, and the conclusion that one or more treatments with a 1:100 dilution produces the best results and the greatest reduction in the intensity of the skin test is likewise confirmed.

In Table 5 are presented seven cases of early hay-fever treated two years in succession. During 1918 and 1919, three patients were treated with grass pollen, during 1919 and 1920 three patients were treated with grass pollen, and during 1917 and 1918 one patient was treated with rose pollen.

Patient 374, who was treated three times with a 1:500 dilution of timothy pollen extract, was 75 per cent benefited, what symptoms this patient had were present chiefly in early June. The second year, although it was found that this patient was as sensitive to the pollen of June grass as to timothy, it was decided to treat him more strenuously with timothy pollen extract again since the first year's treatment with timothy was not deemed sufficient. Following two treatments with the 1:100 dilution of timothy pollen extract the second year, he was again 75 per cent benefited and again his chief symptoms were manifested in June. Therefore, it is quite evident that since his chief symptoms were manifested in early June, previous to timothy pollination and during the pollination of June grass, to which he was very sensitive, that June grass was the cause of the symptoms, and that although timothy treatment had not protected against June grass, it was really successful as far as timothy was concerned. During a third year, 1920, he was treated only with June grass pollen extract and had no symptoms during June grass pollination, but he did have considerable trouble during late June and July. In order to simplify the table this treatment is omitted. Therefore, there is no question that during the 1918 and 1919 seasons his 25 per cent of symptoms were caused by June

TABLE 5—PRESEASONAL TESTS AND TREATMENT WITH GRASS POLLENS TWO SUCCESSIVE YEARS

Pa- tient	Dura- tion	Age of On- set	Season 1918						Season 1919						Result of Treat- ment		
			Tests Before Treatment			No Treat- ments With Final Dilution,	Tests at End of Treatment			Tests Before Treatment			No Treatments With Final Dilution	Tests at End of Treatment			
			Tim- othy	Red Top	June Grass		Tim- othy	Red Top	June Grass	Tim- othy	Red Top	June Grass		Tim- othy		Red Top	June Grass
374	1	47	1-5,000+	1-500+		1-500 3				1-5,000±	1-1,000±	1-5,000±	1-100 2	1-500±	1-1,000±	75%	
375	19	10	1-5,000±	1-500+		1-500 4				1-500±	1-1,000±	1-500±	1-500 4		1-100+		75%
376	22	12	1-5,000±	1-5,000±		1-500 4				1-1,000±	1-1,000±	1-500±	1-100 3		1-100±	1-100+	Good
377	11	8	1-5,000±	1-500+		1-100 1	1-100+			1-5,000±	1-500±	1-5,000±	Season 1920	1-500 4		1-1,000 2	75%
378	25	3	1-5,000±	1-1,000±		1-100 5	1-100±			1-5,000±	1-5,000±	1-5,000±	1-500 4			1-500 1	75%
379	11	3	1-5,000±	1-500+		1-100 3	1-100±	1-500±		1-5,000±	1-1,000+	1-5,000±	1-500 5			1-1,000 3	50%
373	21	3	Rose 1-1,000+	—	—	Rose 1-500 5				Rose 1-1,000±			Season 1918	Rose 1-500 6			Good

grass pollen and that his timothy pollen treatment was successful. Patient 375 was treated four times each year with a 1 500 dilution of timothy pollen alone and was 75 per cent benefited. Since this patient was not very sensitive to June grass and had no symptoms until timothy pollination, it is evident that he had, as already shown, insufficient timothy treatment. Patient 376 was chiefly sensitive to timothy pollen and was free from symptoms following treatment with timothy pollen extract.

Patients 377 and 378 were chiefly sensitive to timothy pollen extract the first year and were free from symptoms following treatment with timothy pollen alone. The second year they were equally sensitive to the pollens of both June grass and timothy, and were given less treatment than the preceding year with timothy pollen and a little treatment with June grass pollen extract, they were only 75 per cent benefited the second year and their symptoms were manifested chiefly in late June and July. Therefore, treatment with the two pollen extracts together diminished the amount of treatment that they might have had with timothy alone, and the result was that both patients were only 75 per cent benefited the second year, furthermore, it is evident that they did not need June grass pollen treatment anyway. The case of Patient 379 was similar to the two cases just discussed, with the exception that this patient was equally sensitive to both pollens the first year as well as the second year, and that she was only 50 per cent benefited the second year by treatment similar to that given the two cases just cited, the fact that this patient began treatment late, thus necessitating a five day interval between treatments, may partly explain the poor results.

Patient 373 was sensitive to the pollen of rose alone, and five and six treatments in 1917 and 1918, respectively, prevented any symptoms both years even though, since she was a nurse in a hospital, she had to be exposed freely to roses. Incidentally, it should be stated that previous to treatment, whenever she scratched her hands on rose thorns, marked itching and swelling resulted at the points of injury, but following rose pollen treatment these symptoms did not occur when injured by rose thorns.

The conclusions to be drawn from Table 5 verify those already proven correct in Table 2. The important conclusions are, that treatment with two pollens together is not advisable, that the early type of hay-fever is chiefly due to timothy pollen, and sufficient treatment with timothy pollen alone, namely, treatment with the 1 100 dilution or its equivalent of a 1 500 dilution, will give satisfactory results in such cases, that this amount of treatment will usually markedly diminish the intensity of the skin test to timothy pollen, and that only an occasional case of early hay-fever is due to rose pollen. The correctness of

TABLE 6—PRESEASONAL TESTS AND TREATMENT WITH RAGWEED POLLEN THREE SUCCESSIVE YEARS

Patient	Age of Onset	Duration	Season 1917				Season 1918				Season 1919				Season 1920			
			Tests Before Treatment	Number of Treatments With Final Dilution	Result of Treatment	Tests Before Treatment	Number of Treatments With Final Dilution	Tests After Treatment	Result of Treatment	Tests Before Treatment	Number of Treatments With Final Dilution	Tests After Treatment	Result of Treatment	Tests Before Treatment	Number of Treatments With Final Dilution	Tests After Treatment	Result of Treatment	
381	38	11	1-1,000±	1-100 1	Good	1-1,000±	1-100 4		Fair	1-1,000±	1-500 4		Fair	1-1,000±	1-500 4		Fair	
382	15	17	1-10,000±	1-500 3	75%	1-10,000±	1-500 3		75%	1-10,000±	1-500 3		75%	1-10,000±	1-500 3		75%	
383	20	10	1-1,000±	1-100 3	Good	1-5,000±	1-500 5		75%	1-1,000±	1-500 5		Good	1-5,000±	1-500 5		Fair	
384	32	10	1-1,000±	1-100 2	Good	1-1,000±	1-500 4		Good	1-1,000±	1-500 4		Good	1-1,000±	1-500 4		Fair	
385	25	8	1-1,000±	1-100 3	Good	1-1,000±	1-100 1		Good	1-1,000±	1-100 1		Good	1-1,000±	1-500 5		Fair	
Season 1919																		
386	9	3	1-1,000±	1-500 3	Good	1-500±	1-100 6	1-100 0	Good	Pol +	1-500 7	1-500 7	Fair	1-500±	1-500 7	1-500 7	Fair	
387	21	5	1-10,000±	1-100 1	75%	1-10,000±	1-100 2	1-100+	Fair	1-5,000±	1-500 5	1-500 5	Fair	1-10,000±	1-500 4	1-500 4	75%	
388	28	15	1-5,000±	1-500 3	50%	1-5,000±	1-100 4	1-100+	Good	1-1,000±	1-500 5	1-500 5	Fair	1-1,000±	1-500 4	1-500 4	75%	
389	17	23	1-5,000±	1-500 2	75%	1-5,000±	1-100 5	1-100±	Fair	1-5,000±	1-500 4	1-500 4	Fair	1-5,000±	1-500 5	1-500 5	75%	
390	34	2	1-1,000±	1-500 5	75%	1-1,000±	1-100 6	1-100+	Good	1-5,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
391	1	34	1-5,000±	1-100 2	75%	1-5,000±	1-100 3	1-100 0	Good	1-1,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
392	20	20	1-5,000±	1-100 2	75%	1-10,000±	1-100 3	1-100 0	Good	1-1,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
393	27	5	1-5,000±	1-500 3	75%	1-5,000±	1-100 3	1-100 0	Good	1-1,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
394	12	20	1-5,000±	1-500 4	Good	1-5,000±	1-100 1	1-500±	Fair	1-5,000±	1-500 4	1-500 4	50%	1-5,000±	1-500 4	1-500 4	50%	
395	11	39	1-5,000±	1-500 3	75%	1-5,000±	1-500 3	1-500±	75%	1-5,000±	1-500 6	1-500 6	Fair	1-5,000±	1-500 6	1-500 6	Fair	
396	21	19	1-5,000±	1-500 4	Good	1-5,000±	1-500 4	1-1,000+	Good	1-1,000±	1-500 4	1-500 4	75%	1-5,000±	1-500 4	1-500 4	75%	
397	24	20	1-5,000±	1-500 5	Fair	1-5,000±	1-100 5	1-100+	Good	1-1,000±	1-500 4	1-500 4	75%	1-5,000±	1-500 4	1-500 4	75%	
398	28	10	1-5,000±	1-500 5	75%	1-5,000±	1-500 5	1-500+	50%	1-5,000±	1-500 4	1-500 4	50%	1-5,000±	1-500 4	1-500 4	50%	
399	30	25	1-10,000±	1-500 6	50%	1-10,000±	1-500 3	1-500+	75%	1-5,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
400	24	10	1-5,000±	1-500 4	50%	1-5,000±	1-500 3	1-100+	75%	1-5,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
401	33	16	1-5,000±	1-500 2	50%	1-5,000±	1-500 5	1-100+	75%	1-5,000±	1-500 5	1-500 5	75%	1-5,000±	1-500 5	1-500 5	75%	
402	26	8	1-10,000±	1-500 3	75%	1-10,000±	1-200 2	1-100+	Fair	1-100+	1-500 2	1-500 2	75%	1-5,000±	1-100 1	1-100 1	75%	
403	16	2	1-10,000±	1-500 2	50%	1-10,000±	1-500 5	1-100+	75%	1-10,000±	1-500 5	1-500 5	75%	1-10,000±	1-100 2	1-100 2	Good	
404	36	6	1-1,000±	1-100 4	Good	1-1,000±	1-100 4	1-100+	Good	1-1,000±	1-100 4	1-100 4	Good	1-1,000±	1-100 4	1-100 4	Good	
405	8	22	1-1,000±	1-100 1	Good	1-1,000±	1-500 4	1-100+	Good	1-500±	1-500 6	1-500 6	Good	1-500±	1-500 6	1-500 6	Good	

the conclusion drawn from Table 4 that usually the patient needs as much treatment with the final dilutions the second year as he received the first year is also proven

In Table 6, twenty-five patients, who were treated three years in succession with ragweed pollen extract, are presented. During 1917 to 1919 inclusive, five patients were treated, and during 1918 to 1920 inclusive, twenty were treated.

Analysis of Table 6 shows that following the first year of treatment, a third of the patients were free from symptoms, one patient, or 4 per cent, was practically free, a third was 75 per cent benefited, and six patients, or 24 per cent, were 50 per cent benefited. Following the second successive year's treatment, ten patients, or 40 per cent, were free from symptoms, seven, or 28 per cent, were practically free, seven, or 28 per cent, were 75 per cent benefited, and one patient, or 4 per cent, was 50 per cent benefited. Following the third successive year's treatment, three patients, or 12 per cent, were free from symptoms, ten, or 40 per cent, were practically free, ten, or 40 per cent, were 75 per cent benefited, and two, or 8 per cent, were 50 per cent benefited. There were no failures in any year. Seven of the twenty-five patients were either entirely free or practically free from symptoms all three years.

A comparison of the results of the three years' treatments with the amount of treatment given to each patient each year substantiates previous conclusions. (1) Treatment with the 1:100 dilution yielded by far the best results. (2) Five or six doses of the 1:500 dilution was followed by excellent results. (3) Three or four doses of the 1:500 dilution sometimes was followed by excellent results but more often by 75 per cent benefit, and (4) two doses of the 1:500 dilution gave poor results.

In twenty-two instances skin tests were done at the end of treatment. Following treatment with the 1:100 dilution of pollen protein, in two instances the 1:100 dilution was negative, in nine instances the 1:100 dilution gave a doubtful to a positive reaction, and in only two instances was the 1:500 dilution at all positive. Following treatment with the 1:500 dilution, in one instance the 1:100 dilution was somewhat positive, in four instances the 1:500 dilution was more or less positive, and in three instances the 1:1,000 dilution was positive. Two of those that were positive with the 1:1,000 dilution were treated four times with the 1:500 dilution, and two of those that were positive with the 1:500 dilution were given only three doses of the 1:500 dilution. Therefore, as shown repeatedly, there is nearly always a great decrease in the sensitivity of the patient, as evidenced by the skin test, following considerable treatment, and the greatest decrease results from treatment with the 1:100 dilution.

TABLE 7—PRESEASONAL TESTS AND TREATMENT WITH GRASS POLLENS THREE SUCCESSIVE SEASONS

Pa- tient	Age of Onset	Dur- a- tion	Season 1918				Season 1919				Season 1920				Result of Treat- ment
			Tests Before Treatment		No Treat- ments With Final Dilu- tion, Tim- othy	Result of Treat- ment	Tests Before Treatment		No Treat- ments With Final Dilu- tion, Tim- othy	Result of Treat- ment	Tests Before Treatment		No Treatments With Final Dilution		
			Tim- othy	Red Top			Tim- othy	Red Top			Tim- othy	Red Top			
410	22	25	1-5,000±	1-1,000+	1-500 3	Fair	1-5,000±	1-5,000±	1-1,000±	1-100 4	Good	1-1,000±	1-1,000±	1-100 3	50%
411	23	10	1-10,000±	1-5,000±	1-500 5	Good	1-10,000±	1-5,000±	1-1,000±	1-100 4	Good	1-1,000+	1-1,000+	1-100 4	75%
412		45	1-500±	1-100±	1-500 6	Fair	1-500±	1-500±	1-100±	1-100 3	Good	1-100±	1-100±	1-500 5	Good
413	15		1-1,000±	1-100+	1-100 3	Good	1-500+	1-500±	1-500±	1-100 1	Good	1-500±	1-500±	1-100 2	Good

In Table 7 are presented four patients with early hay-fever who were treated three years in succession with the grass pollens. The four patients were either free or practically free from symptoms following treatment with timothy pollen alone the first year, and all were entirely free from symptoms following an increased amount of timothy pollen treatment the second year, even though two of the patients were equally sensitive to redtop pollen and quite sensitive to June grass pollen. Therefore, as already proven, timothy pollen treatment will protect against redtop pollen and there was no evidence that the patients needed June grass pollen treatment. From the experience of the first two years, there was every reason to believe that similar timothy pollen treatment alone would ensure excellent results the third year. Therefore, it was decided to make an experiment of these four cases by treating three of them with varying amounts of June grass pollen together with sufficient timothy treatment, and to treat the fourth patient with timothy pollen alone as a control. The results were interesting, in that the first patient who had the maximum treatment with both pollen extracts was only 50 per cent benefited, the second patient who had maximum treatment with timothy pollen, and considerable, though less, treatment with June grass pollen, was 75 per cent benefited, whereas the third patient, who had considerable, though less, treatment with timothy pollen than the other two patients, but who had as much treatment with June grass pollen, was free from symptoms and the fourth patient, following treatment with timothy pollen extract alone, was also free from symptoms. Therefore, the only difference in the treatment of the first three cases between the first two years and the third year was the additional treatment with June grass pollen in the third year. The greater the amount of treatment with June grass pollen extract, the poorer were the results, and the same number of treatments with June grass pollen in a nonsensitive case and no June grass treatment in a nonsensitive case was followed by excellent results. In order to give the first patient three treatments with the 1:100 dilution of pollen extract, this treatment was carried on in increasing large doses during June grass pollination so that the patient was being treated by and at the same time was being exposed to June grass pollen, and the result was that the patient had much hay-fever during June as a result of treatment with large doses of June grass pollen at the time of natural exposure to the pollen, although in the previous two years he either was not sufficiently exposed to June grass pollen or he had sufficient protection against it. The second patient was comparable with the first, with the exception that the June grass pollen treatment was discontinued shortly after the onset of June grass pollination, but timothy pollen treatment was continued as in the first case. Therefore, the second case had less treatment during June grass pollination and

TABLE 8—PRESASONAL TESTS AND TREATMENT WITH RAGWEED POLLEN FOUR SUCCESSIVE YEARS

Patient	Age of Onset	Duration	Season 1917				Season 1918				Season 1919				Season 1920			
			Tests Before Treatment	Number Treatments With Final Dilution	Result of Treatment	Tests Before Treatment	Number Treatments With Final Dilution	Result of Treatment	Tests Before Treatment	Number Treatments With Final Dilution	Result of Treatment	Tests Before Treatment	Number Treatments With Final Dilution	Result of Treatment	Tests Before Treatment	Number Treatments With Final Dilution	Result of Treatment	
403	5	42	1-1,000±	1-100 4	Good	1-100±	1-100 4	75%	1-50+	1-100 4	75%	1-500±	1-100 3	75%	1-500±	1-100 3	75%	
407	31	16	1-1,000±	1-100 3	75%	1-1,000±	1-500 4	Fair	1-1,000±	1-100 2	Good	1-1,000±	1-500 4	Fair	1-1,000±	1-500 4	Fair	
408	21	20	1-5,000±	1-100 3	Fair	1-5,000±	1-500 5	50%	1-5,000±	1-500 5	50%	1-5,000±	1-500 5	75%	1-10,000±	1-500 6	75%	
409	15	45	1-5,000±	1-500 3	50%	1-5,000±	1-500 5	Fair	1-5,000±	1-500 2	None	1-5,000±	1-500 5	Good	1-10,000±	1-500 5	Good	

had less symptoms in June so that the result was 75 per cent benefit. The third patient had as much June grass treatment but was not sensitive to June grass pollen, therefore, June grass treatment during its pollination was harmless and needless as it would have been had it been given to the fourth patient.

The manner in which June grass pollen was given to the patients in Table 7 differs from the way it was given in the preceding tables in that in these cases treatment with June grass was discontinued before the onset of its pollination, whereas, the patients in Table 7 were treated with gradually increasing amounts of June grass pollen during the time the patients were being naturally exposed to the pollen. Therefore, treatment with large amounts of a pollen extract during the season of its pollination is evidently not desirable. The conclusion that has already been repeatedly stated, namely, that mixed pollen treatment is, as a rule, not only unnecessary but undesirable, was equally true of cases in Table 7, and the poorer results obtained in the third successive year of treatment was due, as shown repeatedly, to mistreatment.

In Table 8 are presented four patients who were treated with ragweed pollen extract four successive years.

They illustrate the slightly variable results that may be obtained by giving similar treatment each year to the same patient for several years, and by giving varying amounts of treatment to the same patient for several years.

In three of these cases the results expressed on a percentage basis were practically identical for all four years and the only exception to this in the fourth patient was the unfavorable result that followed too little treatment in the third year. In other words, favorable results may be looked for following any number of successive years' treatment, provided the average number of treatments are given each year. No matter how many successive years the same patient is treated, as a rule, approximately the same amount of treatment with the final dilution is required each year as that which gave desirable results any preceding year, however, no greater amount of treatment is required in successive years than that which gave desirable results any preceding year.

During-the-Season or Curative Treatment with Pollen—Frequently patients present themselves for treatment during their hay-fever attack, and although pollen treatment at this time does not seem to be very logical, on the basis of anaphylaxis, the patient often will insist on taking the chance. Pollen treatment during the season does not seem logical because the patient is being injected with the pollen which is causing symptoms at the same time that he is being exposed to the pollen present in the air which he is inhaling. The danger resulting from large doses of the injected pollen is obvious because of an over-

dose, due to the combination of the injected pollen and the inhaled pollen. Therefore, in order that during-the-season treatment should be beneficial, the patient must be injected with minute amounts of the pollen extract in order to diminish artificially a few of the patient's antibodies, thus leaving a smaller number of antibodies in the patient for combination with the pollen antigen that is inhaled. If too much pollen extract (antigen) is injected, the patient should have symptoms due to over-treatment alone, or he should be made worse, due to the injection of pollen extract (antigen) superimposed on the inhalation of pollen (antigen). It is evident, that on the basis of anaphylaxis, during-the-season treatment is hazardous, and although the skin test is the best guide as to the proper treatment, there is no way of controlling the amount of pollen that the patient may inhale.

TABLE 9—TEST AND TREATMENT DURING SEASON WITH GRASS POLLEN

Patient	Previous No	Tests Before Treatment Timothy	Dates of Treatment	Treatment in Cubic Centimeters With Dilution of Timothy Pollen	Result of Treatment
Season 1917					
415	374	1-20,000±	6/22 to 7/12	1-20,000 = 0.15, 0.3	75%
424		1-1,000±	6/20 to 6/29	1-1,000 = 0.15, 1-500 = 0.1, 0.15	50%
416		1-5,000±	6/22 to 7/14	1-10,000 = 0.15, 1-5,000 = 0.15, 0.3, 0.45, 1-1,000 = 0.15	25%
Season 1919					
440		1-5,000±	7/10 to 7/24	1-10,000 = 0.15, 1-5,000 = 0.15, 0.3	25%
441		1-1,000±	6/10 to 7/14	1-5,000 = 0.1, 0.15, 0.3, 0.45, 1-1,000 = 0.15, 0.3	None
443		1-5,000±	6/16 to 7/10	1-10,000 = 0.15, 0.3, 1-5,000 = 0.15, 0.3, 0.45	Fair
444	208	1-1,000±	6/19 to 7/14	1-5,000 = 0.15, 0.3, 0.45, 1-1,000 = 0.15, 0.3	Fair
445		1-1,000±	6/5 to 7/5	1-5,000 = 0.15, 0.3, 1-1,000 = 0.1, 0.15, 0.3, 0.45	75%
446		1-1,000±	6/16 to 7/14	1-5,000 = 0.15, 0.3, 0.45, 1-1,000 = 0.15, 0.3	75%
442		{ J G 1-500±	5/30 to 6/20	J G 1-1,000 = 0.15, 0.3, 1-500 = 0.15, 0.3	None
Season 1920					
452		1-10,000±	6/15 to 7/6	1-20,000 = 0.15, 1-10,000 = 0.15, 0.3, 0.4	Fair
455		1-100±	6/24 to 7/14	1-1,000 = 0.15, 0.3, 0.3, 0.4, 0.45	75%
456		1-10,000±	6/21 to 7/10	1-20,000 = 0.15, 1-10,000 = 0.15, 0.3, 0.45	50%
457		1-5,000±	6/26 to 7/17	1-10,000 = 0.15, 0.3, 1-5,000 = 0.15, 0.3, 0.45	50%
458		1-5,000±	6/26 to 7/17	1-20,000 = 0.15, 1-10,000 = 0.15, 0.3, 0.45	None
459		1-5,000±	6/24 to 7/17	1-10,000 = 0.15, 0.3, 0.45, 0.55, 0.65	Fair
460		1-5,000±	6/26 to 7/17	1-10,000 = 0.15, 0.3, 0.45, 0.55	None
461		1-1,000±	6/26 to 7/17	1-5,000 = 0.15, 0.3, 0.45, 0.55	25%
462		1-5,000±	6/22 to 7/17	1-10,000 = 0.15, 0.3, 1-5,000 = 0.15, 0.3	None
451		{ T and J G 1-5,000±	6/12 to 7/1	T and J G 1-10,000 = 0.15, 0.3, 0.3, 0.45	Fair
453		{ 1-5,000± J G	5/29 to 6/22	1-20,000 = 0.15, 1-10,000 = 0.15, 0.3, 0.4	Fair
454		{ J G 1-100±	5/29 to 6/16	J G 1-1,000 = 0.15, 0.3, 0.45	Fair

In Table 9 are presented twenty-two patients who were treated with grass pollens during the season of their pollination, and while they were having symptoms from exposure to these pollens. Three patients were treated in 1917 with timothy pollen, in 1919, six were treated with timothy, and one was treated with June grass, and in 1920, nine were treated with timothy, two with the combination of timothy and June grass, and one with June grass alone.

Before considering Table 9, it may be well to explain the notations beneath the headings of each column, using the first patient as an example. Four hundred and fifteen is the sequence number of the patient in the whole series presented in this paper. No 374 in the second column means that this same patient has been presented previously in this paper, and on referring back, it is seen that this patient was presented in Table 5 which consisted of patients who were treated with grass pollen two years in succession, in other words as No 415 the patient was treated during the season in 1917 (Table 9) and preseasonally two successive years, 1918 and 1919 (Table 5). The third column gives the skin test. The next column gives the dates of treatment, that is June 22 to July 12, inclusive. The treatment column gives the dilutions with which the patient was treated and the respective amounts of each treatment dilution in cubic centimeters, for instance 1 20,000 dilution of timothy pollen extract, dose 0.15 c c, then 0.3 c c, after this the 1 10,000 dilution, dose 0.15 and 0.15 c c. The five or seven day interval between doses is understood, and usually the same scheme of treatment was followed as outlined in the first part of this paper for preseasonal treatment, and the skin test was used as the guide to determine the initial dose. The last column gives the result from during-the-season treatment, for instance, the first patient was 75 per cent benefited.

The results from during-the-season treatment were. Of the twenty-two patients treated, none were free from symptoms, seven, or 32 per cent, were practically free, four, or 18 + per cent, were 75 per cent benefited, three, or 13 + per cent, were 50 per cent benefited, the same number were 25 per cent benefited, and five, or 22 + per cent, were not benefited. Since no patients were entirely relieved of symptoms, and since a large percentage were not benefited, it is evident, on comparing these results with those of former tables, that preseasonal treatment ensures better results by far than does during-the-season treatment. If preseasonal treatment is absolutely barred, during-the-season treatment is worth trying, provided it is given with sufficient care and the skin test is used as a guide to the initial treatment.

Patients 442 and 454 were treated with June grass (J G) alone because they were sensitive to this pollen only, and were having symptoms during June grass pollination, as noted by the dates of treatment, namely, from the last of May to the middle of June. Patients 451 and 453 were treated with the combination of timothy and June grass pollen extracts because they were having symptoms and were sensitive to June grass, and they were also sensitive to and had had symptoms during the timothy pollination. The other patients were treated with timothy pollen extract alone because practically all of them were first seen at the end of June grass pollination and at the beginning or during timothy pollination.

With three of the patients we have the opportunity of contrasting during-the-season treatment with preseasonal treatment. Patient 415 was 75 per cent benefited by during the season treatment, and he was similarly benefited by preseasonal treatment in the two following years (Table 5, Patient 374). Patient 445, who was 75 per cent benefited by during-the-season treatment, was free from symptoms following preseasonal treatment (Table 2, Patient 208). Patient 446, who was 75 per cent benefited from during-the-season treatment, was similarly benefited following preseasonal treatment (Table 2, Patient 205), and was not benefited following the same amount of preseasonal treatment (Table 3, Patient 286) as was given during the season. It is not advisable to draw general conclusions from these three cases, although they are of sufficient interest to warrant mention.

TABLE 10—TEST AND TREATMENT DURING SEASON WITH RAGWEED POLLEN

Patient	Previous No	Tests Before Treatment	Dates of Treatment	Treatment in Cubic Centimeters With Dilution of Ragweed Pollen	Result of Treatment
Season 1917					
417	35a	1-1,000+	8/14 to 9/28	1-1,000 = 0 15, 0 2, 0 3, 0 4, 0 45, 0 5	75%
418		1-5,000±	9/ 4 to 9/27	1-10,000 = 0 15, 1-5,000 = 0 15, 1-1,000 = 0 15	
419		1-5,000±	8/27 to 9/ 4	1-10,000 = 0 15, 0 3	
420	36	1-5,000±	9/ 6 to 9/24	1-10,000 = 0 15, 1-5,000 = 0 15, 0 3, 0 45	Fair 50%
421		1-1,000±	8/10 to 9/ 5	1-5,000 = 0 15, 1-1,000 = 15, 3, 1-500 = 0 15, 0 3, 0 45	
422		1-10,000±	9/ 4 to 9/27	1-10,000 = 0 15, 0 3, 1-5,000 = 0 15, 0 3	
423	119	1-5,000±	8/16 to 9/10	1-10,000 = 0 15, 0 3, 1-5,000 = 0 15, 0 3, 0 45	None 50%
Season 1918					
425		1-1,000+	8/17 to 8/29	1-5,000 = 0 15, 0 3, 0 45	
426	372	1-1,000+	8/28 to 9/11	1-5,000 = 0 15, 0 15, 0 2	None
427	6	1-10,000±	8/28 to 9/14	1-20,000 = 0 15, 1-10,000 = 0 15, 0 3	50%
428	59	1-5,000±	8/22 to 9/ 5	1-10,000 = 0 15, 0 3, 0 3	50%
429		1-1,000+	8/20 to 9/14	1-5,000 = 0 15, 0 3, 0 45, 1-1,000 = 0 15, 0 3	None
430		1-1,000±	8/16 to 9/ 7	1-5,000 = 0 15, 0 3, 0 45, 1-1,000 = 0 15	50%
431	61	1-1,000+	8/29 to 9/13	1-5,000 = 0 15, 0 3, 0 45	None
432	346	1-1,000±	8/28 to 9/10	1-5,000 = 0 15, 0 3, 0 45	None
433	369	1-10,000±	8/13 to 8/30	1-20,000 = 0 15, 0 3, 1-10,000 = 0 15	None
434	84	1-1,000+	8/28 to 9/10	1-5,000 = 0 15, 0 3, 0 45	None
435	370	1-1,000+	8/20 to 9/13	1-5,000 = 0 15, 0 3, 0 4, 0 45, 0 5	Good
436		1-1,000±	8/16 to 9/24	1-5,000 = 0 15, 0 3, 0 45, 0 45, 1-1,000 = 0 15, 0 3, 0 3	
437		1-10,000±	8/14 to 9/ 9	1-20,000 = 0 15, 1-10,000 = 0 15, 0 3, 1-5,000 = 0 15, 0 3	
438	184	1-5,000±	8/20 to 9/10	1-10,000 = 0 15, 1-5,000 = 0 15, 0 15, 0 3	None 75%
439		1-5,000±	8/14 to 9/14	1-10,000 = 0 15, 0 3, 1-5,000 = 0 15, 0 3, 0 45	
447		1-10,000+	8/19 to 9/14	1-20,000 = 0 15, 0 3, 1-10,000 = 0 15, 0 3, 0 45	
448	62	1-10,000±	9/ 1 to 9/21	1-20,000 = 0 15, 0 3, 1-10,000 = 0 15, 0 3	None
449		1-5,000±	8/29 to 9/11	1-10,000 = 0 15, 0 3, 1-5,000 = 0 15	None
450		1-10,000±	7/31 to 9/21	1-20,000 = 0 15, 0 3, 0 45, 0 55, 1-5,000 = 0 15, 0 3, 0 45, 0 55	50%
463		1-1,000+	8/20 to 9/20	1-5,000 = 0 15, 0 3, 0 45, 1-1,000 = 0 15, 0 3	Good

In Table 10 are presented twenty-seven patients who were treated with ragweed pollen extract during the season. The notations in this table are exactly like those in Table 9, therefore, no explanation is needed. It should be stated, however, that these patients were tested with and found to be either not sensitive or only slightly sensitive to other pollens.

Of the twenty-seven patients presented in Table 10, three, or 11 + per cent, were free from symptoms, one patient, or 3 + per cent, was practically free, three patients, or 11+ per cent, were 75 per cent benefited, seven, or 25+ per cent, were 50 per cent benefited, one patient, or 3+ per cent, was 25 per cent benefited, and twelve patients, or 44+ per cent, were not benefited. Therefore, although a few patients were free from symptoms, and as many more were 75 per cent benefited, the number who were only 50 per cent benefited was as great as the number who were more than 50 per cent benefited, and nearly half of the whole series were not benefited at all. On comparing these results from during-the-season treatment with the results presented in former tables from preseasonal treatment, it is evident that for late hay-fever by far the best results are obtained from preseasonal treatment, and it is questionable whether during-the-season treatment is even worth giving when it is taken into consideration that there are localities to which patients may go where ragweed does not exist.

Since fifteen of these patients have been discussed elsewhere in this paper, it is possible with these to compare during-the-season treatment with preseasonal treatment. Ten of the patients were presented in Table 1, therefore, it is possible to contrast the results from the two kinds of treatment in these cases with little detail. Patient 417 (35a) was free from symptoms following preseasonal treatment and 75 per cent benefited from during-the-season treatment, Patient 419 (36) was free from symptoms following preseasonal and practically free from symptoms from during-the-season treatment, Patient 428 (59) was 50 per cent benefited as a result of both kinds of treatment, therefore, in these three cases, the results from preseasonal treatment were slightly better than those from during-the-season treatment in two of them, and the same in the third case. The other seven cases in this group, namely, Patients 422 (119), 427 (6), 431 (61), 435 (84), 439 (184), 447 (10) and 449 (62) were not benefited by during-the-season treatment, but following preseasonal treatment three were free from symptoms, one was practically free, two were 75 per cent benefited and the remaining patient was 50 per cent benefited. Therefore, in these seven cases during-the-season treatment was a failure, whereas preseasonal treatment gave very satisfactory results. Since the remaining five patients in Table 10, that have been presented elsewhere, were all presented in Table 4, they may be considered together with little detail. Patient 426 (372) was 50 per cent benefited by during-the-season treatment, and was 50 per cent and 75 per cent, respectively, benefited the two seasons during which he was pre-seasonally treated, Patient 435 (370) was free from symptoms from during-the-season treatment and was free from symptoms and 75 per

cent benefited the two respective seasons following preseasonal treatment, therefore, in these two cases, the results from both kinds of treatment were very similar. Patients 425 (371), 432 (346) were not benefited at all by during-the-season treatment, whereas following pre-seasonal treatment two successive years, Patient 425 (371) was free from symptoms and 75 per cent benefited, Patient 432 (346) was 75 per cent benefited both years. Patient 433 (369) was practically free and 75 per cent benefited. With these three cases, therefore, during-the-season treatment was a failure, whereas preseasonal treatment was very satisfactory.

From these fifteen patients who were treated both ways it may be noted that frequently during-the-season treatment gives as good results as preseasonal treatment. This was true in five, or one third, of the cases. However, more often during-the-season treatment is a failure in the same cases in which preseasonal treatment is very satisfactory. This was true in ten, or two-thirds, of the cases. These conclusions also verify what was stated in the second preceeding paragraph, namely, that for late hay-fever by far the best results are obtained from pre-seasonal treatment than from during-the-season treatment.

In Table 11 are presented nineteen patients who were treated with bacterial vaccines during their hay-fever attack, although they were very sensitive to ragweed pollen and were having hay-fever symptoms during ragweed pollination. The reason for such treatment was that it is quite possible that ragweed pollen exposure may in some cases cause such a severe irritation of the mucous membranes that ever-present bacteria may, either alone or together with ragweed pollen, be a cause of hay-fever symptoms. In our study on pollen asthmatics⁸ this was found to be the case in many instances, and Frank and Strouse,⁹ Medalia,¹⁰ Scheppegrell¹¹ and others, have considered this to be true in hay-fever cases. The construction of Table 11 differs from that of Tables 9 and 10 in only two ways, namely, that treatment was given with bacterial vaccines, and that the numerals under the treatment column represent that many hundred millions of bacteria per dose. In some instances, the vaccine used was an autogenous one made from the patient's nasal secretions, in other instances it was a stock vaccine consisting of *Staphylococcus pyogenus aureus* or *S. albus*, and for those patients who were treated in 1920, a mixed

8 Walker, I. C. Sensitization and Treatment of Bronchial Asthmatics with Pollens, *Am J M Sc* **157** 409, 1919

9 Frank, I., and Strouse, S. Pollen Extracts and Bacterial Vaccines in Hay-Fever *J A M A* **72** 1593 (May 31) 1919

10 Medalia, L. S. Hay-Fever, Its Treatment with Autogenous Vaccines and Pollen Extract, *Boston M & S J* **175** 201, 1916

11 Scheppegrell, W. Anaphylaxis Due to Pollen Protein, with a Report of the Results of Treatment in the Hay-Fever Clinic of the New Orleans Charity Hospital *Laryngoscope* **28** 853, 1919

streptococcus vaccine was used. This mixed streptococcus vaccine consisted of equal amounts of the six streptococci that were most frequently found in the sputum and nasal secretions of asthmatic patients.¹² These streptococci, according to Holman's classification, were hemolytic types *S. pyogenes*, *S. infrequens* and *S. anginosus* and nonhemolytic types *S. salivarius*, *S. ignavus* and *S. mitis* in the proportion of approximately sixteen million of each per one hundred million of total, so that a dose of 300 million mixed streptococci represented about fifty millions of each of the six strains.

TABLE 11—DURING SEASON TREATMENT WITH BACTERIA

Patient	Pre-vious No	Skin Test With Ragweed	Dates of Treatment	Treatment With Vaccines	Each Treatment Recorded in Hundred Million	Result of Treatment
Season 1919						
464	{ 184	1-5,000±	8/30 to 9/15	Autog nasal	3, 4, 5	Good
	{ 439					
465	(264	1-10,000±	8/30 to 9/15	Staph pyog albus	3, 4, 5	None
	{ 181					
466		1-5,000±	9/ 6 to 9/14	Autog nasal	3, 4, 5	50%
467	272	1-5,000±	9/10 to 9/24	Autog nasal	3, 4, 5	75%
468	11	1-10,000±	8/23 to 9/13	Staph pyog albus	3, 4, 5	None
469		1-10,000±	9/ 5 to 9/20	Autog nasal	3, 4, 5	None
470	185	1-5,000±	8/23 to 9/12	Staph pyog aureus	3, 4, 5, 6	75%
471	385	1-1,000±	8/29 to 9/18	Autog nasal and alb	3, 4, 5, 6	None
472	167	1-10,000±	8/20 to 9/18	Autog strept	3, 4, 5, 6	Good
Season 1920						
473	463	1-1,000+	9/ 4 to 9/20	Mixed strept	3, 4, 5	None
474		1-5,000+	8/30 to 9/20	Mixed strept	3, 4, 5	50%
475		1-1,000+	9/ 6 to 9/20	Mixed strept	3, 4, 5	None
476	10	1-10,000±	8/23 to 9/20	Mixed strept	3, 4, 5, 6, 7	None
477		1-5,000±	8/23 to 9/13	Mixed strept	3, 4, 5, 6	25%
478		1-500+	8/30 to 9/20	Mixed strept	3, 4, 5, 6	50%
479		1-1,000+	9/ 1 to 9/20	Mixed strept	3, 4, 5	None
480	186	1-10,000±	8/30 to 9/20	Mixed strept	3, 4, 5, 6	Fair
481	102	1-5,000±	9/ 4 to 9/11	Mixed strept	3, 4	50%
482	154	1-5,000+	9/ 4 to 9/11	Mixed strept	3, 4	Fair

Since Patients 466, 469, 474, 475, 477, 478 and 479 had not been previously observed or treated by me, their cases will be considered together and not too much stress will be placed on the results. Patient 466 was 50 per cent benefited and Patient 469 was not benefited by treatment with autogenous vaccines made from the nasal secretions. Patients 474 and 478 were 50 per cent benefited, and Patient 477 was 25 per cent benefited by the mixed streptococcus vaccine. Patients 475 and 479 were not benefited by the mixed streptococcus vaccine. The only conclusion to be drawn from these cases is that sometimes an autogenous nasal vaccine or a mixed streptococcus vaccine given during the hay-fever attack benefits somewhat an occasional patient but just as often this treatment fails.

¹² Walker, I. C., and Adkinson, J. Types of Streptococci Found in the Sputum of Bronchial Asthmatics, J. M. Research 40 229, 1919

The twelve following patients, however, since they were treated with ragweed pollen either during or preceeding the ragweed season in some year, give more definite information. Patients 464 (184 and 439) and 472 (187) were relieved of symptoms while being treated with their autogenous nasal secretion vaccine, whereas both patients were only 50 per cent benefited from preseasonal pollen treatment (Table 1, Patients 184 and 187) and one patient was not benefited at all by during-the-season pollen treatment (Table 10, Patient 439). In these two control cases it may be assumed that bacteria played some part, at least, in the cause of symptoms. Since Patients 468 (11), 471 (385), 473 (463) and 476 (10) were not benefited by vaccine treatment but all had been free from symptoms from ragweed pollen treatment, it is certain that bacteria played no part in the causation of their hay-fever symptoms. These four patients had been treated with ragweed pollen extract. Patients 468 (11) and 476 (10), preseasonal one year and were free from symptoms (Table 1, Nos 10 and 11), Patients 471 (385), preseasonal three years in succession and was free from symptoms two years and practically free the third year (Table 6, No 385), Patient 473 (463) treated during the season was free from symptoms (Table 10, No 462). Patient 465 who was not benefited by either *Staphylococcus pyogenes albus* vaccine or by too little pre-seasonal treatment (Table 3, No 264), and was only 50 per cent benefited by considerable preseasonal treatment (Table 1, No 181) gives no information with the exception that *Staphylococcus pyogenes albus* played no part in the causation of hay-fever symptoms in this case, this same organism was used in two cases already discussed, Patients 468 and 471, with no benefit. Patients 480 (186) and 482 (154) both became practically free from symptoms during mixed streptococcus vaccine treatment, whereas from preseasonal pollen treatment both were benefited only 75 per cent (Table 1, Nos 186 and 154), therefore, in these two cases streptococci probably played a considerable part in the causation of hay-fever symptoms. Patient 470 (185) was 75 per cent benefited during treatment with *Staphylococcus pyogenes aureus* vaccine, whereas only a 50 per cent benefit followed preseasonal pollen treatment (Table 1, No 185), therefore *Staphylococcus pyogenes aureus* probably played some part in the causation of hay-fever symptoms in this case. Patient 481 (108) was 50 per cent benefited during mixed streptococcus vaccine treatment, but was not improved by preseasonal pollen treatment (Table 1, No 108), therefore, in this case it is quite evident that streptococci played a part in the causation of hay-fever symptoms.

The series of patients who were treated with bacterial vaccines is too small to justify sweeping conclusions, however, it does seem as though treatment with bacterial vaccines were beneficial for a few

hay-fever patients who are very sensitive to pollen. If a vaccine is to be used, the choice of bacteria would seem to be either an autogenous nasal or a mixed streptococcus vaccine. In the few cases in which considerable preseasonal pollen treatment is not very satisfactory, it is worth while to try during-the-season treatment with autogenous nasal secretion or a mixed streptococcus vaccine.

In Table 12 are presented eighteen patients who were treated with ragweed pollen both preceding and during the season. The first nine patients were given the usual preseasonal pollen treatment and then this treatment was discontinued. A little later, during the same season, these patients reported that they were having more or less hay-fever so that during-the-season treatment with pollens was then given as described in Table 10, in other words, the two methods of treatment were given to these patients. The other nine patients were treated with gradually increasing doses of the pollen extract, but the treatment

TABLE 12—PRECEDING AND DURING SEASON TREATMENT
WITH RAGWEED POLLEN

Patient	Pre vious No	Tests Before Treatment	Dates of Treatment	Treatment in Cubic Centimeters With Dilution of Ragweed Pollen	Result of Treatment
483	14	1-1,000±	8/21 to 9/10	1-5,000 = 0 15, 0 3, 0 45, 0 55	50%
484	308	1-1,000±	8/23 to 9/10	1-5,000 = 0 15, 0 3, 0 45	Same
485	18	1-5,000±	8/22 to 9/ 3	1-5,000 = 0 1, 0 2, 0 3	Same
486	314	1-5,000±	8/21 to 9/10	1-10,000 = 0 15, 0 25, 1-5,000 = 0 15, 0 25	None
487	399	1-10,000±	8/26 to 9/10	1-10,000 = 0 15, 1-5,000 = 0 1, 0 2	Worse
488	317	1-1,000±	8/20 to 9/ 3	1-1,000 = 0 15, 1-500 = 0 15, 0 15	Same
489	33	1-5,000±	8/22 to 9/18	1-10,000 = 0 15, 0 25, 0 35, 1-5,000 = 0 2, 0 2	50%
490	320	1-5,000±	8/28 to 9/10	1-10,000 = 0 15, 0 25, 0 35	None
491	35	1-5,000±	8/21 to 9/10	1-5,000 = 0 15, 0 25, 0 35	Same
492		1-5,000±	7/18 to 9/10	Usual treatment including 1-500 = 0 55	None
493		1-10,000±	7/ 7 to 9/20	Usual treatment including 1-500 = 0 55	50%
494		1-20,000±	6/30 to 9/13	Usual treatment including 1-500 = 0 65	75%
495		1-10,000±	6/14 to 9/ 4	Usual treatment including 1-500 = 0 25	75%
496		1-5,000±	7/ 3 to 9/13	Usual treatment including 1-500 = 0 25	75%
497	469	1-5,000±	7/10 to 9/20	Usual treatment including 1-500 = 0 25	50%
498		1-10,000±	7/ 3 to 9/ 4	Usual treatment including 1-500 = 0 45	Fair
499		1-5,000±	7/ 3 to 9/ 4	Usual treatment including 1-500 = 0 3	50%
500		1-10,000±	7/10 to 9/20	Usual treatment including 1-500 = 0 25	50%

was begun so late preseasonally that not enough treatment would have been given had treatment been discontinued at the onset of pollination, therefore, the usual preseasonal treatment, using gradually increasing doses, was continued on through the season of pollination. For example, Patient 492 was treated with gradually increasing amounts of ragweed pollen extract from July 18 to September 10 inclusive; the usual preseasonal treatment was begun preseasonally and continued into or through the season of pollination.

Patients 483 and 489 were not benefited by preseasonal treatment (Table 1, Nos 14 and 33), they were then given during-the-season treatment, as indicated in Table 12, with the result that they were 50 per cent benefited. Patients 485 and 491 were benefited 75 and 50 per cent, respectively, following preseasonal treatment (Table 1,

Nos 18 and 35), they were then given during-the-season treatment, as indicated in Table 12, with no apparent change in their symptoms. Patients 484, 486, 488 and 490 were all unimproved by during-the-season treatment following the preseasonal treatment (as indicated in Table 4, Nos 308, 314, 317 and 320) which in two cases failed to benefit and in the two others there was a 50 per cent benefit. The remaining patient (487) was not as much benefited while being treated during the season as he had been by preseasonal treatment (Table 6, No 399). Therefore, in only two of these nine cases did during-the-season treatment improve the results already obtained from preseasonal treatment, but since these two patients were not benefited by preseasonal treatment (Table 1, Nos 14 and 33) and became 50 per cent benefited by during-the-season treatment, the latter treatment was worth trying after the former had failed, in other words, two failures were changed to 50 per cent benefit.

The remaining nine patients were treated according to the pre-seasonal schedule, but treatment was begun very late (in July) and continued into the hay-fever season. Although this is the first time this method of pollen treatment has been mentioned in this paper, it has been used extensively in the past by many investigators. The results from this method of treatment, as indicated in Table 12, show that no patients were entirely free from symptoms, one patient, or 11 per cent, was practically free, one third of the patients were 75 per cent benefited, 44 per cent were 50 per cent benefited, and one patient, or 11 per cent, was not benefited. Of course, this series of cases is much too small to warrant conclusions, yet it would seem fair to say that this method of treatment is preferable to during-the-season treatment alone but not as desirable as the regular preseasonal treatment which is begun early enough to permit of its discontinuance just prior to the season of pollination.

Hay-Fever Caused By the Pollen of Trees—So far, we have been concerned with the two pollen seasons, namely, April and May (ragweed) and May to August (the grasses). *aphylo-* is a third and earlier pollen season which begins in March and continues into June during which time various trees pollinate. Before maple often pollinates in February, the other maples, birches, the alder and hazel nut pollinate in late March or early April, the *Juniper*, cottonwood and elm pollinate during April, the oaks, bayberry and hickory pollinate during May, the pines pollinate in late May and early June, and the fruit trees pollinate in May. I have observed twelve patients who were sensitive to and had hay-fever from the pollen of trees. One patient had hay-fever caused by the pollen of apple blossoms, and he was free from symptoms following preseasonal treatment with its pollen extract. One patient, who was

sensitive to the pollen of the oak and maple, and another, who was sensitive to the pollen of the willow, were both free from symptoms following preseasonal treatment with these pollen extracts. Patients not treated were those sensitive to the following tree pollens: one patient, to poplar tree pollen, one, to pine tree pollen, two, to ash, one, to willow, two, to both willow and poplar, and one to willow, poplar and ash. Since the season of pollination of the individual trees continues only from a few days to two weeks at the most, it does not seem essential that treatment be given.

Other Pollens that May Cause Hay-Fever —It has been shown in this paper that the chief causes of hay-fever occurring in the New England States were the pollens of ragweed, timothy and June grass, that an occasional cause is the pollen of the rose and redtop grass and of various trees. There is, however, almost an unlimited list of pollens that may at any time, but probably rarely, cause hay-fever. Therefore, for those who treat a large number of hay-fever patients and for those who fail to obtain satisfactory results from treatment with the common pollens mentioned, it is advisable to have a very extensive assortment of pollens. As a rule, however, it would seem to be sufficient to warn the ragweed patients that they should not smell of golden rod, golden glow, sunflower, poppy, aster, chrysanthemum and the like that pollinate during the ragweed season. The grass cases may be warned to avoid close contact with clover, lilies, daisy, dandelion, rose, lawn grass, orchard grass, corn and the like that pollinate during the grass season. In certain localities in this country it may be necessary to treat with the pollen extracts of the various grains, with sunflower or what not but this necessity is not universal and is limited to the various localities where exposure to these particular pollens is unavoidable.

Other Parts of Plants that May Cause Hay-Fever —The leaves of certain plants and trees may cause hay-fever. The under surface of leaves has a fine hairy growth, and these fine hairs may cause hay-fever. I have observed one patient who had hay-fever from and was sensitive to the hairs of the willow tree leaf, treatment with an extract of these leaves prevented hay-fever. Another patient was sensitized to and had hay-fever from the plantain leaf. It is quite probable that the plane tree, the common shade tree in London, may cause hay-fever. Such instances, however, are extremely rare and should not be considered, as a rule.

Foods May Cause Seasonal Hay-Fever —Seasonal hay-fever, due primarily to foods, has not come to my notice. However, during their season many hay-fever patients find that certain foods aggravate their symptoms, whereas these same foods may be eaten without symptoms at a time when they are not accustomed to have hay-fever,

and they can eat the foods during their hay-fever season, provided they have had sufficient pollen treatment to protect them against the particular pollen that primarily causes their hay-fever. These foods are usually the fruits, and they have no apparent relationship to the causative pollen. Among the usual fruits should be mentioned peach, melon and apple. Green corn and the use of beer and wine often aggravate ragweed hay-fever, but they do not aggravate grass pollen hay-fever as one would expect to be the case, celery sometimes acts similarly. With the peach it is often the skin that causes trouble, whereas the pulp usually does not. The cooking of the fruits often renders them inert.

Animal Emanations May Cause Seasonal Hay-Fever—The patient who is sensitive to animal emanations usually has symptoms on exposure to the animal at any and all times of the year, occasionally, however, such a patient has symptoms only from exposure to the animal during the spring and summer, when the animal is shedding, moulting or perspiring profusely.

Bacteria May Cause Seasonal Hay-Fever—The possibility of bacterial infection as a complication of or secondary to pollen sensitization has already been discussed, and the possibility of bacterial infection as a primary cause of hay-fever in patients who are not sensitive to pollens must be considered. Each year I examine three or four patients who have seasonal hay-fever, but who are not sensitive to any of the pollens, therefore, these patients are treated with their autogenous nasal secretion vaccine.

REPORT OF CASES

CASE 1—L. W., a woman, aged 35, has had hay-fever from August first to the end of October for three years. Her symptoms are sneezing, running of the nose and some wheezing.

Not only were the skin tests with pollens negative, but she also failed to give an ophthalmic test, and snuffing the whole pollen of ragweed and dropping a concentrated solution of ragweed pollen extract into her nose failed to provoke symptoms.

The patient was treated with an autogenous vaccine, made from her nasal secretion, in gradually increasing amounts, beginning with 300 million streptococci, from June 25 to Oct 11, 1919. At the end of the season, she reported that in previous summers she had always lost three weeks of sleep, whereas during the past summer she had lost only one week of sleep, and that her days had been quite free from symptoms.

In June, 1920, she reported that her hay-fever had begun about three weeks previously. She was again treated as previously, from June 7 to July 12, when she discontinued treatment because she had been entirely free from symptoms for the past three weeks and she continued to be free during the summer.

CASE 2—H. S. M., a woman, aged 29, has had hay-fever for five years, from August 15 to the first frost. The symptoms are sneezing, tickling in the throat and running of the nose, alternating with stuffing up of the nose. She was first seen in June, 1918, and her symptoms had already begun this year, much earlier than usual.

She was treated with gradually increasing amounts of an autogenous vaccine made from her nasal secretion, from June 21 to August 14, the first treatment was 300 million streptococci. In the winter following this treatment, she reported that she had had only six bad days of hay-fever during the whole summer, she claimed that she had been markedly free from hay-fever.

The patient returned the following year, in May, 1919, and reported that her sneezing and running of the nose had just begun again. She was treated again with a new autogenous vaccine in a similar manner as was done the preceding year. During the first six weeks of treatment, she had occasional attacks of hay-fever, but not as bad as usual, during the remainder of the summer she was practically free from hay-fever.

CASE 3—E. L. T., a woman, aged 30, has had hay-fever for four years, from August to the first frost. Her hay-fever is worse at night and prevents sleep. Her present attack began August 26. She was not sensitive to pollens.

She was treated from September 1 to October 6, inclusive, with an autogenous nasal secretion vaccine consisting of *Staphylococcus pyogenes aureus* in gradually increasing amounts, beginning with 300 million bacteria, during the season, she was given treatment with vaccines. Following the first treatment, she was a little better, and following each succeeding treatment she was free from symptoms for four days but had considerable hay-fever the two days preceding each treatment.

These three patients would seem to prove that there are cases of seasonal hay-fever not caused by pollens but caused by bacteria and that treatment with autogenous vaccines made from their nasal secretions is often very effective. It is noted that in these nonsensitive pollen cases of hay-fever there was an absence of eye symptoms, as noted in a previous paper¹³.

Hay-Fever Caused By Olfactory Irritants or Pseudo-Hay-Fever — Goodale¹⁴ called attention to the fact that during pollen seasons certain individuals who are sensitive to and have hay-fever from pollens also have vaso-motor symptoms ranging from sneezing to asthmatic attacks due to the fragrance of certain heavily scented flowers that have no pollen or to which pollen the individual is not sensitive. He considers that the symptoms are reflex and that the path of transmission is along the olfactory nerve. The flowers most commonly responsible are lilies, hyacinths, sweet peas, lilacs, honey suckle and peonies.

I classify the causes of symptoms as mechanical, chemical, odorific and thermal. Among the mechanical causes any kind of dust is the most frequent cause, more especially sweeping dust and hay dust, fine powder, such as talcum and the like, is also a frequent cause. Among the chemical irritants, soap powder, lye and ammoniacal fumes are very frequent causes. Among the odorific irritants, heavily scented perfumes, face powders, musty air and stable odors are frequent causes. Thermal irritants concern sudden changes of temperature,

¹³ Walker, I. C. Frequent Causes and the Treatment of Perennial Hay-Fever, J. A. M. A. **75** 782 (Sept 18) 1920.

¹⁴ Goodale, J. L. The Present Status of Immunization in Hay-Fever, Boston M. & S. J. **179** 293, 1918.

as in going from warm air to extreme cold, from moist air to very dry air, and exposure to drafts, a very frequent history is that of a paroxysm of sneezing with or without running of the nose on retiring and arising. The mechanism seems to be a reflex due to the sudden exposure of the warm and protected skin of the body to cold air, as in getting out of bed and in undressing, during which acts the warm body surface is suddenly and momentarily exposed to cool air, in other words, there is a mild chilling of the body surface.¹⁵ The same mechanism holds for many who take cold easily. Appropriate pollen treatment for those who are sensitive usually relieves these symptoms.

TABLE 13—PRESEASONAL TREATMENT WITH RAGWEED POLLEN EXTRACT

Results	1917		1918		1919		1920		Total	
	No of Patients	Percentage of Total Patients	No of Patients	Percentage of Total Patients	No of Patients	Percentage of Total Patients	No of Patients	Percentage of Total Patients	No of Patients	Percentage of Total Patients
Good	19	70	26	32	52	37	13	7	110	25
Fair	1	4	9	11	32	23	48	25	90	20
75%	4	15	23	28	31	22	82	43	140	32
50%	3	11	16	20	15	10	45	23	79	18
None	0	0	7	8½	10	7	3	1½	20	4½
Total	27		81		140		191		439	

B Preseasonal Treatment with Grass Pollens

Good	8	80	10	66	13	72	5	14	36	46
Fair	0	0	3	20	1	5	7	20	11	14
75%	1	10	2	13	2	11	12	33	17	21 8
50%	1	10	0	0	2	11	8	23	11	14
None	0	0	0	0	0	0	3	8½	3	4
Total	10		15		18		35		78	

SUMMARY

The methods used by me in testing and treating seasonal hay-fever and the seasons of pollination of the causative pollens are described in detail. Eight tables are then presented to illustrate the benefits obtained by preseasonal pollen treatment. Table 3 shows the unsatisfactory results obtained by giving less than three doses of the 1:500 dilution of the pollen extract, and the other seven tables show the good results obtained when more than three doses of the 1:500 dilution, or preferably one or more treatments with the 1:100 dilution of the pollen extract are given. Tables 1 and 2 deal with a single season's treatment, and Tables 4 to 8 inclusive, deal with two or more successive seasons' treatment. In general, the greater the amount of treatment given the better the symptomatic results and the greater is the reduction

¹⁵ Mudd, S., and Grant, S. B. Reactions to Chilling of the Body Surface, *J. M. Research* 40: 53, 1919.

in the positiveness of the skin test • Table 13 is a composite of Table 1 to 8 inclusive, with the exception of Table 3 which concerns insufficient treatment. It shows the results obtained from preseasonal treatment with ragweed pollen and with the grass pollens for each year and the results for all four years.

The percentage of results varies somewhat for different years, this is due, in great part, to variation in treatment. The average results for all four years following preseasonal ragweed treatment show that nearly 50 per cent of the patients had little or no symptoms, and a third of the others were 75 per cent benefited, whereas in only 4.5 per cent of all of the cases was there failure from treatment. In some of the individual years, treatment showed much better results than this. The average results of treatment with the grass pollen group of cases shows that 60 per cent of the patients had little or no symptoms, and 21.8 per cent were 75 per cent benefited, whereas 4 per cent were not benefited. In some of the individual years, treatment gave much better results than in other years, and this variation was due to varying treatment. Unless patients return for future treatment, it is very difficult to get in touch with them in order to find out the permanency of relief from treatment. In four cases, however, it has been possible to learn that more or less permanency of benefit follows sufficient preseasonal treatment. Case 11, treated in 1917, was also free from symptoms in 1918 and 1919 without any treatment, but in 1920 this patient had severe symptoms. Case 1 was free from symptoms following treatment but has not been heard from since. Case 70 was free from symptoms following treatment in 1919, and was also free from symptoms in 1920, without treatment. Case 312, free from symptoms in 1918 and 1919 following treatment both years, had no symptoms in 1920 without treatment.

Tables 9 and 10 deal with during-the-season pollen treatment, and the general conclusion is that during-the-season pollen treatment with the grasses is worth doing but is not as satisfactory as the preseasonal treatment, and that during-the-season treatment with ragweed pollen is very unsatisfactory. Treatment for the early type of hay-fever seems to yield better results than treatment for the late type. This is probably due to the fact that there are several causes for the early type of hay-fever such as June grass, clover and timothy pollen all of which pollinate at different times and each over a short interval and it is usually only one of these pollens that cause symptoms. Therefore, the actual cause of early hay-fever is present a short time, whereas ragweed which is practically always the only cause of late hay-fever pollinates as long a time as all of the early pollens together, and a much longer time than any one of the early pollens.

Table 11 presents pollen sensitive patients who were treated with bacterial vaccines, and there is evidence that bacteria may, in some pollen sensitive cases, play a part in the cause of hay-fever symptoms. With those patients who are not sufficiently benefited by supposedly satisfactory preseasonal pollen treatment, treatment with autogenous nasal vaccines is indicated during the pollen season.

Table 12 presents cases treated with ragweed pollen preceding and during the season. In the case of patients not much benefited by satisfactory preseasonal pollen treatment, small doses of the pollen protein given during the season may produce benefit. In the case of those patients who present themselves too late for sufficient pre-seasonal treatment, gradually increasing amounts of the pollen protein may be given throughout the hay-fever season. The results from this kind of treatment seem to be better than those from during-the-season treatment alone with either pollens or bacteria but not nearly as satisfactory as the results obtained from preseasonal pollen treatment that is begun early enough to permit of its discontinuance before the season of pollination.

Since close contact with pollens that are closely related to the causative pollen, the eating of fruits, and certain olfactory irritants may all complicate the cause and the treatment of hay-fever, it is not remarkable that hay-fever treatment does not always afford entire protection.

Other causes of seasonal hay-fever that must be considered are tree pollens, animal emanation and bacteria.

CONCLUSIONS

Satisfactory preseasonal pollen treatment yields excellent results in seasonal hay-fever. By satisfactory treatment is meant five or six treatments with a 1:500 dilution of the pollen extract, or, better still, two or three treatments with a 1:100 dilution of the pollen extract.

When preseasonal pollen treatment fails, sometimes benefit results from during-the-season treatment with pollens and sometimes from during-the-season treatment with an autogenous nasal secretion vaccine. During the season treatment with pollens without preseasonal treatment is not very satisfactory although such treatment for the early type of hay-fever is worth doing provided for some reason or other it is not possible to give preseasonal treatment.

Treatment that of necessity must begin late preseasonally may be continued on through the pollen season with better results than those obtained by during-the-season treatment alone, but with much less

beneficial results than those obtained by beginning preseasonal treatment early enough to permit of its discontinuance before the season of pollination begins

Although in the New England States the pollen of ragweed (dwarf variety) is practically always the cause of late hay-fever (August and September), and the pollen of timothy grass is the cause of probably 90 per cent of the early hay-fever (June and July), in other localities other pollens may play a great part in the cause of either type of hay-fever. For this reason it is essential that other observers publish their tests, results and treatment in detail in order to learn the causative pollens and proper treatment in various localities

In the New England States the pollen of rose and redtop grass occasionally cause early hay-fever, and treatment occasionally has to be given with these. The pollen of June grass is a more or less common cause of early hay-fever but when the season of its pollination is very early, unless the treatment is begun very early, too little treatment can be given with it to be of benefit, and when its pollination is very late sufficient treatment with timothy pollen has been given to protect against June grass pollen exposure

Treatment with a combination of timothy and June grass pollens was not successful in my hands because of insufficient treatment with both pollens, the addition of June grass pollen retarded the amount of treatment that otherwise would have been given with timothy alone. Another illustration of the undesirability of mixed pollen treatment is noted with those patients who have both the early and the late types of hay-fever. In these cases, during the month of May and part of June, large amounts of timothy pollen extract should be given together with small amounts of ragweed pollen extract, and the result in these cases is rather poor because there is a tendency to restrict the timothy treatment for fear of producing anaphylaxis from the combination of two pollens to which the patient is sensitive. In other words, treatment with a combination of pollens either diminishes the amount of treatment that is required with one or all of them or pushing the treatment with the combination leads to the danger of anaphylaxis. For either or both of these reasons, I see no benefit to be derived from mixed pollen therapy. As regards the early type of hay-fever, I consider it best to treat preseasonally with timothy or the chief causative pollen and, if necessary to treat during the season with June grass pollen

Since intimate exposure to other pollens which may be attributing causes of hay-fever, the eating of fruits during the hay-fever season, the possibility that bacteria play a part in the cause of hay-fever and

the exposure to olfactory irritants may all aggravate the symptoms of hay-fever, it is not remarkable that hay-fever treatment is not perfect ¹⁶

16 Selfridge,¹⁷ Koessler¹⁸ and Scheppegrell¹⁹ have published the prevailing pollens in their respective localities, namely, California, Illinois and the Southern States and they have designated those pollens that seem to be the chief causes of hay-fever in these localities. It is desirable that others in various localities do likewise.

17 Selfridge, G. Spasmodic Vasomotor Disturbances of the Respiratory Tract, with Special Reference to Hay-Fever, California State J M **16** 164, 1918.

18 Koessler, K. K. The Specific Treatment of Hay-Fever (Pollen Disease), Billings-Forchheimer Therapeutics of Internal Diseases, **5** New York, D Appleton & Co.

19 Scheppegrell, W. Hay-Fever in the Southern States, Southern M J **9** 614, 1916.

SOME URINARY CHANGES IN NORMAL INDIVIDUALS ON THE PELLAGRA PRODUCING EXPERIMENTAL DIET *

M X SULLIVAN
SPARTANBURG, S C

Many of the pellagra patients admitted to the Pellagra Hospital in 1917 gave a history of using diets which consisted mainly of cereals, fat pork, molasses and greens, with little, if any, animal protein. On such a type of diet, Goldberger and Wheeler¹ experimentally produced pellagra in the human subject at the Rankin Farm, Mississippi State Penitentiary. To test the pellagra producing diet as regards its effect on the main urinary constituents, six normal people were placed on this Rankin Farm experimental diet as given for the week ending Aug 8, 1915. It was intended to run the experiment for at least several weeks. Unfortunately, on the fifth day of the period of the experimental diet, the water supply of the laboratory froze and the experiment was brought to an end. Accordingly, only the data for four or five days, on the diet mentioned, are available, but these data are of some interest. The diet used was as follows:

BILL OF FARE, WEEK ENDING AUG 9, 1915

August 2

Breakfast	Biscuits, fried mush, grits and brown gravy, sirup, coffee with sugar
Dinner	Corn bread, cabbage, sweet potatoes, grits, sirup
Supper	Fried mush, biscuits, rice, gravy, cane sirup, coffee, sugar

August 3

Breakfast	Biscuits, mush, rice, gravy, sirup, coffee, sugar
Dinner	Corn bread, collards, sweet potatoes, grits, sirup
Supper	Biscuits, mush, grits, gravy, sirup, coffee, sugar

August 4

Breakfast	Biscuits, mush, grits, gravy, sirup, coffee, sugar
Dinner	Corn bread, collards, sweet potatoes, rice, sirup
Supper	Biscuits, mush, grits, gravy, sirup, coffee and sugar

August 5

Breakfast	Biscuits, mush, grits, gravy, sirup, coffee, sugar
Dinner	Corn bread, collards, sweet potatoes, grits, sirup
Supper	Biscuits, mush, rice, gravy, sirup, coffee, sugar

August 6

Breakfast	Biscuits, mush, rice, gravy, sirup, coffee, sugar
Dinner	Corn bread, collards, sweet potatoes, grits, sirup
Supper	Biscuits, mush, grits, gravy, sirup, coffee, sugar

* From the Pellagra Hospital, U S Public Health Service

1 Public Health Rep 30 No 46, Nov 13, 1915, Bull 120, Hyg Lab, Washington, D C

TABLE 1—URINARY DATA OF NORMAL PEOPLE ON THE PELLAGRA PRODUCING EXPERIMENTAL DIET

Sub ject	Weight kg	Date	Diet	Volume, Cc	Specific Gravity	Total Nitrogen, Gm	Ammonia Nitrogen Gm	Urea, Gm	Urea Nitrogen, Gm	Creat inin, Gm	Creatinin Nitrogen Gm	Urea N per Cent of Total Nitrogen	NHAN per Cent of Total Nitrogen
1	76.2	Dec 2-3	Normal	770	1.025	8.410	0.316	13.749	6.416	1.247	0.464	76.29	3.76
		Dec 3-4	Mixed	1,410	1.019	11.822	0.436	21.429	10.000	1.503	0.559	84.59	3.69
		Dec 4-5	Diet	1,600	1.016	9.973	0.432	18.433	8.602	1.379	0.513	86.25	4.33
		Dec 5-6	Diet										
1		Dec 6-7	Rankin farm pellagra producing diet	690	1.031	7.403	0.352	11.602	5.414	1.118	0.416	73.13	4.75
		Dec 7-8	Rankin farm pellagra producing diet	540	1.033	5.972	0.462	8.872	4.140	1.067	0.397	69.32	7.74
		Dec 8-9	Rankin farm pellagra producing diet	600	1.031	6.196	0.437	9.206	4.324	1.034	0.384	69.78	7.05
		Dec 9-10	Rankin farm pellagra producing diet	690	1.032	6.248	0.419	8.057	3.760			60.18	6.71
2	52.6 52.6 52.6 52.7	Dec 10-11	Rankin farm pellagra producing diet	510	1.024	5.622	0.502	6.585	3.073			54.66	8.93
		Dec 2-3	Normal	1,650	1.018	10.831	0.346	20.148	9.402	1.285	0.478	80.81	3.19
		Dec 3-4	Normal	1,400	1.018	12.433	0.459	20.199	9.426	1.418	0.527	75.81	3.69
		Dec 4-5	Normal	930	1.025	11.414	0.513	20.593	9.610	1.236	0.467	84.19	4.49
2	51.4	Dec 5-6	Normal	1,440	1.022	12.500	0.512	23.510	10.971	1.341	0.498	87.14	4.07
		Dec 6-7	Pellagra producing diet	1,190	1.020	8.304	0.391	14.497	6.765	1.252	0.465	81.47	4.71
		Dec 7-8	Pellagra producing diet	1,160	1.020	5.759	0.347	9.056	4.226	1.342	0.499	73.38	6.02
		Dec 8-9	Pellagra producing diet	1,125	1.013	5.447	0.339	8.734	4.076	1.125	0.418	74.83	6.22
3	63.7	Dec 9-10	Pellagra producing diet	700	1.026	5.984	0.356	9.281	4.331			72.38	5.95
		Dec 10-11	Pellagra producing diet	750	1.023	6.319	0.357						5.65
		Dec 2-3	Normal	1,080	1.029	11.408	0.521	19.046	8.888	1.286	0.478	77.91	4.57
		Dec 3-4	Normal	1,160	1.028	11.421	0.499	19.494	9.097	1.305	0.485	79.65	4.37
3	63.3	Dec 4-5	Normal	1,670	1.020	12.300	0.509	21.607	10.083	1.470	0.546	81.98	4.87
		Dec 5-6	Normal	1,560	1.021	12.585	0.581	24.225	11.305	1.487	0.553	89.83	4.62
		Dec 6-7	Pellagra producing diet	920	1.032	8.805	0.472	15.547	7.255	1.096	0.407	82.40	5.36
		Dec 7-8	Pellagra producing diet	950	1.028	6.917	0.448	12.337	5.757	1.304	0.485	83.23	6.48
3	63.3	Dec 8-9	Pellagra producing diet	1,275	1.017	6.313	0.460	9.587	4.471			70.87	7.29
		Dec 9-10	Pellagra producing diet	800	1.029	6.302	0.530	8.276	3.862			61.28	8.41
3		Dec 10-11	Pellagra producing diet	875	1.023	7.735	0.436	8.488	3.961			51.09	5.62

4	53 9	Dec 3-4 Dec 5-6	Normal Normal	910 1,360	1 025 1 011	10 292 7 277	0 310 0 225	16 479 13 406	7 690 6 256	1 384 0 918	0 514 0 341	74 71 85 97	3 20 3 09
4	54 4	Dec 6-7 Dec 7-8 Dec 8-9 Dec 9-10	Pellagra producing diet Pellagra producing diet Pellagra producing diet Pellagra producing diet	1,470 900 1,185 1,010	1 026 1 023 1 016 1 012	6 548 6 280 6 764 5 007	0 284 0 362 0 317 0 302	11 413 11 379 11 465 7 710	5 326 5 310 5 350 3 598	1 117 1 218 1 002	0 527 0 475 0 372	81 34 84 55 79 10 71 86	4 31 5 76 4 54 6 03
		Dec 10-11	Pellagra producing diet	600	1 027	5 433	0 318	6 879	3 210			59 09	5 85
5	49 3	Dec 3-4 Dec 1-5 Dec 5-6	Normal Normal Normal	885 1,210 815	1 023 1 021 1 031	9 959 11 383 - 13 511	0 464 0 581 0 566	16 209 26 392 20 705	7 544 12 316 9 602	0 885 1 167 1 031	0 329 0 434 0 383	75 95 85 63 71 51	4 66 4 04 4 19
	48 5	Dec 6-7 Dec 7-8 Dec 8-9 Dec 9-10	Pellagra producing diet Pellagra producing diet Pellagra producing diet Pellagra producing diet	1,270 1,125 1,675 1,010	1 022 1 024 1 010 1 023	9 355 5 647 4 720 5 037	0 389 0 369 0 348 0 276	17 199 8 839 8 250 7 423	8 026 4 125 3 850 3 464	0 989 0 930 0 969	0 308 0 346 0 300	85 79 73 05 81 57 68 77	4 16 6 53 7 37 5 48
6	56 9	Dec 2-3 Dec 3-4 Dec 4-5 Dec 5-6	Normal Normal Normal Normal	990 700 575 590	1 028 1 034 1 033 1 035	9 434 7 529 8 127 7 908	0 448 0 367 0 394 0 443	15 628 11 490 13 018 13 185	7 293 5 362 6 075 6 293	1 572 1 232 1 109 1 036	0 584 0 458 0 412 0 404	77 31 71 22 74 75 79 58	4 75 4 85 4 85 5 60
6	57 0	Dec 6-7 Dec 7-8 Dec 8-9 Dec 9-10	Pellagra producing diet Pellagra producing diet Pellagra producing diet Pellagra producing diet	690 1,190 1,415 1,200	1 034 1 021 1 014 1 016	7 412 6 761 6 039 5 679	0 457 0 532 0 447 0 381	12 385 12 780 7 929 8 237	6 013 5 964 3 700 3 844	1 118 1 220 0 980	0 416 0 453 0 361	81 13 88 21 61 27 67 69	6 17 7 87 7 57 6 71
		Dec 10-11	Pellagra producing diet	560	1 028	4 565	0 285	5 803	2 708			59 32	6 24

TABLE 2 — URINE DATA OF NORMAL PEOPLE ON THE RANKIN FARM PELLAGRA PRODUCING EXPERIMENTAL DIET

	Volume, C c	Specific Gravity	Total Nitrogen Gm	Ammonia Nitrogen, Gm	Urea Nitrogen Gm	Creatinin, Gm	Creatinin Nitrogen, Gm	Urea N per Cent of Total Nitrogen	NH ₄ N per Cent of Total Nitrogen	Average Weight, Kg	Creatinin Coeffi- cient
Diet Dec 26											
Normal mixed diet, average of 20 tests	1,137	1 024	10 680	0 452	8 616	1 268	0 471	80 35	4 23	59 1	7.88
Pellagra diet, average 24 tests Dec 6 10	1,042	1 023	6 454	0 395	4 873	1 132	0 421	74 86	6 22		
Pellagra diet, average of 1st test after 4 days on the diet	807	1 023	5 710	0 377	3 810	1 069 (3d day)	0 397 (3d day)	67 03	6 55	58 3	7 05
Average for 26 pellagra patients in active pel- lagra, severe, moderate, mild cases	1,158	1 020	6 214	0 519	4 477	0 751	0 279	71 81	8 453	47 46	5 82
Average of same 26 pa- tients, cured	1,420	1 020	10 588	0 605	8 665	0 856	0 318	81 64	5 79	49 397	6 34

August 7

Breakfast	Biscuits, mush, grits, gravy, sirup, coffee, sugar
Dinner	Corn bread, collards, sweet potatoes, rice, sirup
Supper	Biscuits, mush, grits, gravy, sirup, coffee, sugar

August 8

Breakfast	Biscuits, mush, grits, gravy, sirup, coffee, sugar
Dinner	Corn bread, collards, sweet potatoes, grits, sirup
Supper	Biscuits, mush, rice, gravy, sirup, coffee, sugar

Each person was allowed to eat as much of the diet as he or she pleased. With one exception, Subject 1, all the volunteers were males.

The methods of analysis employed were for total nitrogen, Kjeldahl, for ammonia, Folin's² method as modified by Steel³, creatinin by Folin's method⁴, urea by Folin and Denis' method,⁵ using Jack bean as a source of urease and a reaction temperature of from 50 to 55 C.

The individual records for volume, specific gravity, total nitrogen, ammonia nitrogen, urea and urea nitrogen, creatinin and creatinin nitrogen, the ratio of urea nitrogen and ammonia nitrogen to total nitrogen are given in Table 1, and the average for the fourth day on the pellagra producing experimental diet are given in Table 2. By reference to these tables, it will be noted that on changing from the ordinary mixed diet to the Rankin Farm pellagra producing diet there was a marked change in the urine. The average volume decreased, the total nitrogen and urea nitrogen decreased markedly, the urea nitrogen especially, the ammonia decreased slightly. The ratio of urea nitrogen to total nitrogen was markedly lowered, the ammonia ratio increased.

The findings recorded for the fourth day on the diet are comparable to those which obtain in the urine of pellagra patients in the active stage of the disease,⁶ especially for total nitrogen, urea, and, to a degree, for ammonia and are such as would obtain on a low protein diet as found by Folin.⁷

Many thanks are due the volunteers on this diet and especially to Mr. R. E. Stanton for his assistance.

² Am J Physiol **13** 45, 1905

³ J Biol Chem **8** 365, 1910

⁴ Ztschr f Physiol Chem **41** 223, 1904

⁵ J Biol Chem **26** 501, 1916

⁶ Sullivan, Stanton, and Dawson. Metabolism in Pellagra. A Study of the Urine. Archives Intern Med **27** 387, 1921

⁷ Laws Governing the Chemical Composition of Urine. Am J Physiol **13** 67, 1905

Book Reviews

TUBERCULOSIS OF CHILDREN, ITS DIAGNOSIS AND TREATMENT By HANS MUCH, M D, translated from the German by MAX ROTHSCHILD, M D Ed 1 Pp 156 New York The Macmillan Company, 1921

This volume contains very little as to the diagnosis of tuberculosis, but emphasizes particularly the types of immunity and immunologic reactions in tuberculosis and gives a new method of treatment, based largely on the use of "partial antigens" Many new ideas are advanced, which make the book most interesting The author adheres to the old idea that erythema nodosum is a tuberculid, to which the reviewer takes exception The translator, at least, makes the common mistake of using the words "tubercular" and "tuberculous" synonymously The book should be read by all students of immunology and by those particularly interested in the treatment of tuberculosis

Fifty cents each will be paid for the following issues of the Archives of Internal Medicine January, March, June, August, 1918 January and July, 1916, November, 1915, January, 1911, July, 1909 AMERICAN MEDICAL ASSOCIATION, 535 North Dearborn Street, Chicago, Ill

OBJECTS AND METHOD OF DIET ADJUSTMENT IN DIABETES ¹

R T WOODYATT, M D
CHICAGO

In the dietetic management of diabetes we are engaged in the effort to correlate symptoms and signs shown by the patient with the kinds and quantities of food he consumes. The success of treatment, the average of results in all types of cases, depends on the truth of our concept of the relationship existing between symptoms or signs and the food supply. During the last few years the average of results obtained in the dietetic management of diabetes has been improved greatly through the work of Allen and Joslin, and the system they have developed is in some respects more logical and less empirical than any we have had heretofore. Yet the literature of the subject is still confused by a lack of unanimity among all writers as to the best manner of handling all cases. In a recent monograph, Falta ¹ has again told the merits of his "cereal cure" (Mehlfruchtekur), and endorsed methods of management that differ materially from that which has found so much favor in this country. Newburgh and Marsh,² failing to achieve practical results by their application of the principles of "total dietary restriction" and "undernutrition," resort to low protein high fat diets with striking success in the management of seventy-four cases. In the past, good results were obtained and may still be obtained in some cases by old fashioned "rigid" diets. The remarkable improvements that have sometimes been seen with the institution of a Donkin "milk cure," a von Duering "rice cure," a Mossé "potato cure," a von Noorden "oatmeal cure" or any one of several analogous procedures cannot be denied and have never been explained fully to the extent that one may predict with certainty just when one of these empirical procedures will and when it will not produce a result better than that attainable by a more systematic method. The present study was made in the effort to correlate some of these varying views of different writers, and, if possible, to clear away some of the confusion that they tend to create in the mind of the physician as to what method of procedure he may best follow in any specific situation.

¹From the Otho S. A. Sprague Memorial Institute Laboratory for Clinical Research, Rush Medical College.

¹ Falta, *Die Mehlfruchtekur bei Diabetes Mellitus*, Berlin, 1920.

² Newburgh and Marsh, *Arch. Int. Med.* **25**: 647 (May) 1920.

Discrepancies in the clinical literature of diabetes arise from three main sources (1) Confusion in the minds of writers as to the exact nature of the anomaly of the metabolism which characterizes diabetes (2) A general tendency among clinicians to think of the food supply of the body too exclusively in terms of diet, to the neglect of endogenous factors, and (3) the almost universal custom of thinking of the food supply simply as so much carbohydrate, so much protein, so much fat and so many calories without further analysis

It will be necessary to develop each of these points separately

NATURE OF THE DIABETIC ANOMALY AND PRINCIPLES OF TREATMENT

(a) One single specific defect characterizes diabetes This consists *in an inability on the part of the body to utilize as much glucose as may be utilized by the normal body when the supply of glucose exceeds certain limits* The diabetic appears to be capable of utilizing a limited quantity of glucose as well as the normal individual, but fails to utilize a normal percentage of any glucose introduced into the body in excess of this limit This limitation of the body's power to utilize glucose is present in every case of diabetes In its absence we can not say that diabetes exists In its presence alone, without any other accompaniment, one must say that diabetes does exist

(b) The endocrine function of the pancreas, so far as we know it at all, is a single highly selective function having to do with the utilization of glucose and nothing else All that we know of this function is deduced from our knowledge of the changes which occur in the diabetic metabolism and from the established relation between diabetes and the pancreas Were it not for the occurrence of the metabolic phenomenon which is called diabetes, we would have no more reason to speak of a special endocrine function of the pancreas than we now have for speaking of such a function in the case of any of the organs which are not considered as endocrine organs in the usual sense of the word No second metabolic disturbance besides diabetes has been found to occur with consistency after reduction of pancreatic substance It is accordingly necessary to conclude that the work of the pancreas as an endocrine organ, so far as we know it today, has to do solely with the disposition of glucose, and that to lessen this work, one must lessen the quantity of glucose entering the metabolism The pancreas is a glandular organ innervated from the celiac plexus Vagus and sympathetic fibers pass into its substance It is a nerve gland apparatus Presumably it performs its function by secreting a substance having the power to dissociate the one specific sugar glucose into which all other carbohydrates that are capable of utilization on the large scale must first be converted before they can be oxidized, reduced or built up into glycogen That such an apparatus should be stimulated by glucose to

provide for the disposition of glucose is a thought in keeping with many facts That a weakened pancreas in the presence of a sufficient supply of glucose might be stimulated into a state of fatigue and decreased function, and that a sufficient diminution of the glucose supply might then lessen the stimulation, place the organ in a state of comparative rest, and permit it to recuperate up to a certain limit, is also quite conceivable and affords a logical explanation of many observed facts

(c) The anomaly of the metabolism in which abnormal quantities of acetone, acetoacetic, and β hydroxybutyric acids appear in the tissues, blood and urine is not due directly to any impairment of the endocrine function of the pancreas It is a secondary effect in the nature of a disturbed metabolic balance resulting from the withdrawal of oxidizing glucose, as, when one stone is withdrawn from an arch all the other members may settle to a new position This anomaly is not peculiar to diabetes nor constantly associated with it It occurs in other diseases It may be made to appear in a normal subject by starvation, or a diet containing too low a proportion of carbohydrate and too high a proportion of fat, and when this is done it may be made to disappear again simply by the addition of more carbohydrate to the diet It appears to be the immediate result of the oxidation of certain fatty acids in the absence of a sufficient proportion of "oxidizing" (dissociated) glucose

There is for any given individual at any given time, a definite ratio between the quantity of glucose oxidizing in the body and the maximum quantity of ketogenic fatty acids that can be oxidized in the same time without the appearance of abnormal amounts of the acetone bodies In other words, the quantity of oxidizing glucose fixes an upper limit to the quantity of ketogenic fatty acid that can be completely oxidized at the same time As to the absolute magnitude of this ratio, and the degrees of its variation in different individuals in health and disease, it is too early to make a final statement Some years ago, on the basis of chemical studies by Ciamicran and Silber, and test tube experiments with acetoacetic acid, I suggested a certain type of reaction as the basis of "antiketogenesis" in which one molecule of acetoacetic acid would react with one molecule of an alcohol or glucose³ Zeller, working with normal individuals on ample diets consisting of carbohydrate and fat with very low protein contents, shifted the proportions of fat and carbohydrate without changing the total calories and saw acetone appear when the ratio of carbohydrate calories fell below 10 per cent of the total, that is, when the ratio of fat to carbohydrate in the diet in grams was about 4 to 1 Recalculating Zeller's experiments, Lusk estimated the relative quantities of sugar and higher fatty acid

³ Woodyatt, R T J A M A 55 2109 (Dec 19) 1910

that might have been oxidizing together in the body. Allowing for the formation of sugar from the glycerol of the fat, and of glucose from the protein catabolized, and for some glucose from glycogen, but not for ketogenic amino-acids from protein, Lusk suggested that possibly one triose molecule was necessary for the complete oxidation of one of higher fatty acid, that is one molecule of glucose to two molecules of higher fatty acid, or 3 gm to 1 gm. P. A. Shaffer⁴ has worked with test tube experiments and with diabetic individuals in which he conducted metabolism studies, including observations on the respiratory quotient at the time acetone first appeared. Shaffer calculated the ketogenic acids of protein on the basis of the quantities of leucin, tyrosin and phenyl alanin found by analysis in 100 gm of ox muscle protein by Osborne, assuming that each molecule of these known acetone formers may yield one molecule of acetoacetic acid, or its equivalent. As a result of his work, Shaffer suggests that one molecule of glucose is necessary for the complete oxidation of one molecule of acetoacetic acid, or one molecule of any higher fatty or amino-acid that yields one molecule of acetoacetic acid (or equivalent). The molecular weight of glucose being 180, of oleic acid 284, of palmitic acid 256 and the average of the two acids 270, the ratio found by Shaffer if expressed in grains would be about 1.5 gm higher fatty acid to 1 gm glucose. Working with diabetic patients on maintenance diets under conditions that made it probable that the proportion of food stuffs in the diets corresponded fairly with those actually catabolized in the body, and estimating the glucose and fatty acid as hereinafter indicated, we have also observed at the time acetone appeared, ratios of 1.5 or a little above or below this figure with considerable frequency in harmony with the work of Shaffer. Accordingly even though it may prove necessary to correct this figure as data accumulate, and for individual cases it would seem that for clinical purposes one will make no gross error if it is assumed that the ratio of higher fatty acids to glucose, which if exceeded will lead to acidosis, is likely to be close to 1.5 to 1 (in gm). This refers to the materials actually catabolized and to the diet only under the stated conditions.

TREATMENT

It follows from the foregoing that the rationale of dietetic management in diabetes is *to bring the quantity of glucose entering the metabolism from all sources below the quantity that can be utilized without abnormal waste, and to adjust the supply of fatty acids in relationship to the quantity of glucose* so that in the mixture of food stuffs oxidizing in the body, the ratio of the ketogenic fatty acids to

⁴ Shaffer P. A. Proc. Am. Soc. Biol. Chem. Fifteenth Annual Meeting December 1920 p. 6. I. Biol. Chem. 46:98 (March) 1921.

glucose shall not exceed limits compatible with freedom from ketonuria. When, as, and if, under these conditions of relative rest for the pancreas, the glucose using function improves, then the food supply may be increased gradually in so far as this can be done without disturbing the above relations.

ENDOGENOUS FACTORS OF FOOD SUPPLY, ILLOGICAL DIET RESTRICTIONS

When a man fasts he does not, of course, cease to produce heat. Normal men during a fast on light exertion have been observed to produce from 29 to 30 calories per kg of body weight daily. For a 50 kg man this would mean 1,500 calories per day. During the first four days of a fast, Cetti produced on the average 29 calories per kg for a total of 1,618 calories per day, and catabolized 85.88 gm protein for 329.8 calories and 136.72 gm fat for the remaining 1,288 calories.⁵ In a case studied by F. G. Benedict,⁶ on the second day of fasting there were produced 1,768 calories, or 29.9 calories per kg, and the individual was estimated to have catabolized 74.7 gm protein, 147.5 gm fat and 23.1 gm glycogen. These figures are by no means the highest that might be selected. Thus, a well nourished normal man weighing 50 kg, who during a fast produces 1,500 calories per day, may actually catabolize in the neighborhood of 75 gm protein, 125 gm fat and a little carbohydrate from glycogen. These well known facts are repeated simply to emphasize the magnitude of the food supply from the tissues in fasting, and to point out, in particular, that in fasting over 100 gm fat may be thrown into the metabolic stream and catabolized daily. It has further been shown that the amount of fat in the fasting organism materially affects the amount of protein burned. In a critical review of the literature of the subject, Lusk has said: "Where there was much fat present, little protein was consumed, when there was little fat, much protein burned, and when there was no fat, protein alone yielded the energy for life." In a normal individual, the ingestion of fat will not prevent the death of the organism because there is a continual loss of tissue protein from the body which finally weakens some vital organ to such an extent that death takes place. But the ingestion of fat may spare tissue fat and thus prevent the protein loss from becoming abnormally great. It may be said that the ingestion of fat spares the individual any such protein loss as will occur if the tissue fat is allowed to become too much depleted. In this sense, the ingestion of fat by an emaciated individual spares protein for that individual. Voit found in a fasting animal that the ingestion of suitable amounts of fat scarcely influenced the protein metabolism. To one dog "which in starvation

⁵ Citation from Lusk, *Elements of the Science of Nutrition*, Ed. 3, 1917, pp. 86-89.

⁶ Benedict, F. G., *The Influence of Inanition on Metabolism*, 1907, p. 194, Table 128.

burned 96 gm fat, Voit gave 100 gm fat with the result that it burned 97 gm." The fat ingested simply burned instead of the body fat, but the total amount of protein and fat burned remained the same" (Lusk)

Now, if a certain diabetic patient during a fast reacts essentially as a nondiabetic individual in the same state of nutrition, and if he weighs 50 kg, produces from 1,250 to 1,500 calories, and in doing so actually mobilizes and burns 100 to 120 gm, or more, fat, the ingestion of an equal quantity of fat should leave his metabolism in the same state as before. The supply of fat would come at one time from the tissues, at another from the diet, but the quantity thrown into metabolism—the quantity presenting itself for disposition in the cells would be the same in both cases. *If these premises are sound, why then should we ever use complete fasting for diabetes?* Granting that a diabetic patient may suffer from obesity as well as diabetes—that he may have two different metabolic defects, or granting that at times it might seem desirable to starve for other conditions besides diabetes. In such cases fasting would be rational, if it would improve the general condition. But for diabetes itself and particularly for diabetes associated with undernutrition, why for the purpose of desugarization should the patient be compelled to draw from his tissues the fat that he might draw from a diet, especially if in drawing from his tissues he lowers his fat reserves to the extent that he increases his protein losses? The striking results that have been obtained by Newburgh and Marsh with fat replacement diets bear significantly on this point. The practice of starving, or virtually starving, a patient in order to render his urine sugar "free," and then building up the diet, first with carbohydrate and then with protein, with a particular avoidance of fat, would appear to be based on the supposition that if fat were administered it would increase the catabolism of fat. But this would be in disregard of the endogenous food supply, and illustrates the necessity of thinking in terms of the metabolism rather than of the diet. As the diet falls, the endogenous supply rises to take its place, and vice versa. The lower the diet, the less its significance in calculating the food supply from all sources. It is possible to maintain the normal body with a diet that contains only 10 per cent more calories than are produced in fasting, and the differences in the catabolism of a man when receiving no diet and when receiving a 1,500 calorie diet may be slight.

(3) DEALING WITH THE FOOD SUPPLY IN TERMS OF CARBOHYDRATE PROTEIN AND FAT

Carbohydrate, protein and fat are as such three separate and distinct substances, no one of which can be expressed quantitatively in terms of another, and if we speak of food supplies or diets as made up of so

much carbohydrate, so much protein and so much fat, we simply name them in terms of three variables. Thus each particular combination or diet becomes a specific, and having learned by experience how one of them will affect a certain patient, we have no means of knowing exactly how a second dissimilar combination will affect the same patient, much less another, except by trial and experience. The number of possible combinations of three variables is infinite and the number of practical food combinations, no two of which will differ by less than 5 gm of one ingredient or by less than 50 calories runs into the thousands. Accordingly, if we attempt to correlate symptoms and signs shown by the patient with the diet and follow the usual system of thinking of diets simply in terms of carbohydrate protein and fat, without attempting to resolve them into simpler terms, it will be necessary to establish by experiment the effects of each diet combination in every type of case. It would seem tedious and hopeless to proceed by this inductive method. A further objection to this method, and a clear advantage in using another, lies in the fact that protein, carbohydrate and fat as such are not in reality the substances that present themselves for the final oxidative attack in the body which results in the liberation of energy. These substances are resolved by the processes of digestion and intermediary metabolism into simpler substances before they can be burned in the tissues. It is not starch in the bowel nor glycogen in the liver and muscles that taxes the endocrine function of the pancreas, but the glucose into which these carbohydrates are resolved. Protein of the diet ceases to be protein and becomes a mixture of amino-acids before it can be absorbed from the bowel and these undergo deaminations, etc prior to actual oxidation. Neutral fats, to be sure, may be absorbed in part as such, and may be deposited in the tissues as such, but before they can be oxidized and used as sources of energy, they must first be saponified into glycerol and higher fatty acids. Thus, as a matter of fact, carbohydrate protein and fat are not the actual food stuffs with which we are dealing when it comes to the final metabolic processes. In the management of the diabetic food supply, it is simpler and more rational to think in terms of the chemical metabolism. Falta devised a formula in which he added the carbohydrate of the diet to the urinary nitrogen times 28, or (following Lusk's suggestion) 3.65 to show the total quantity of glucose entering the body from carbohydrate and protein. Then, subtracting from this the quantity of glucose excreted in the urine, he obtained a figure for the quantity of glucose actually utilized. The quantity excreted, divided by the total quantity, supplied gives a fraction which multiplied by 100 was Falta's diabetic quotient. The latter has a limited value. The absolute quantity utilized is of more interest. The principle is important and can be developed further.

WHAT FOODS BECOME IN THE BODY

Of the carbohydrates of the diet and tissues that play a significant rôle in heat production, all that do not already exist in the form of glucose are converted into this form by the processes of digestion or intermediate metabolism or both prior to ultimate utilization. We may, therefore, say that 100 gm. utilizable carbohydrate in the diet if all is digested and absorbed, introduce into the metabolism about 100 gm. glucose.⁷

Fats of the diet in the course of digestion and intermediate metabolism must be saponified into glycerol and higher fatty acid before they can be oxidized. Fats such as tristearin, or an oil such as triolein, when completely saponified, yield approximately ten parts by weight of glycerol to ninety parts by weight of higher fatty acid. Glycerol is capable of conversion into glucose in the body almost gram for gram. So we may say, for clinical purposes, that 100 gm. mixed fat in the diet, if completely absorbed and catabolized, will introduce into the metabolism about 10 gm. glucose and 90 gm. higher fatty acid.

Protein of the diet, or tissues, is resolved into amino-acids, and in so far as these are absorbed and catabolized, they must be deaminized (and presumably at the same time oxidized) to yield oxy- or hydroxy acids. Of these, a part is convertible into glucose, another part into β hydroxybutyric and acetoacetic acids, while a third small fraction is destroyed in as yet unknown ways. According to the experiments of Lusk on phlorhizinized dogs, which have been repeated by others, and according to numerous observations in cases of maximal human diabetes, one may say that 100 gm. mixed food protein are capable of introducing into the metabolism approximately 58 gm. glucose. The same 100 gm. of protein also introduce a certain quantity of products which are quite the equivalent of products of the higher fatty acid catabolism in that they are capable of yielding β hydroxy and acetoacetic acids. The exact quantity of these substances formed in the catabolism of 100 gm. protein can be estimated only roughly. The amino-acids that are certainly known to yield acetone bodies are leucin, tyrosin and phenyl alanin. If we take the quantities of these amino-

7 In tables of food analyses all that is listed as carbohydrate is not necessarily utilizable carbohydrate.

8 If objection is made to the view that neutral fats may yield 10 per cent of their weight in glucose on the grounds that ingested neutral fats have not been observed to cause the appearance of "extra" sugar in the urine of phlorhizinized dogs, it may be suggested that the ingestion of fat by a starving phlorhizinized dog would scarcely be expected to increase the fat catabolism for reasons already given unless special precaution had been taken to discharge tissue fat, etc. It is not improbable that the tissue fat that a fasting phlorhizinized dog catabolizes does produce glucose and that the glucose is then credited to protein.

acids found in 100 gm ox muscle protein by Osborne and Mendel,⁹ as Shaffer has also done, and convert the weights given into gram molecules, we obtain 0.16 gm molecules of these ketogenic amino-acids. If we assume that each of these is capable of yielding one molecule of acetoacetic, or β hydroxybutyric acid, and accept the view that one molecule of a higher fatty acid such as oleic or palmitic acid also yields one molecule of aceto-acetic (or β hydroxybutyric acid, then the 0.16 gm molecule of ketogenic amino-acids would be equivalent in respect of its ability to form aceto-acetic acid to 0.16 gm molecule of higher fatty acid. If the acid were oleic (molecular weight 284), this would mean $\frac{45.44}{284}$ gm. The nature of this estimation is such that considerable error is scarcely avoidable, but it affords a tentative figure that serves a practical purpose, even though it may require correction in the light of experience.

It will thus be seen that all of the foods of the diet, except a small fraction of the protein, resolve themselves in the body into two things, glucose, on the one hand, and higher fatty acid (or acetone forming equivalents) on the other. If we let G stand for the quantity of glucose, and F A for the quantity of higher fatty acids that may be introduced into the metabolism by a mixture of carbohydrate, protein and fat, then

100 gm carbohydrate yields in the body	100 gm G and 0 gm FA
100 gm protein yields in the body	58 gm G and 46 gm (?) FA
100 gm fat yields in the body	10 gm G and 90 gm FA

These relationships may be expressed in the form of simple equations in which G is the total quantity of glucose introduced into the body by a given food combination, F A the total quantity of higher fatty acids (plus ketogenic amino-acids expressed in terms of higher fatty acid) C carbohydrate, P, protein, and F, fat (neutral), thus

$$(1) \quad G = C + 0.58 P + 0.1 F$$

$$(2) \quad F A = 0.46 P + 0.9 F$$

If the ratio of F A / G which if exceeded leads to acetonuria is 1.5 / 1, then when $\frac{F A}{G} = 1.5$ we derive from (1) and (2) the equation

$F = 2C + 5.4 P$, which for clinical purposes may be stated simply as

$$(3) \quad F = 2C + \frac{P}{2}$$

DISCUSSION OF HYPOTHETICAL DIETS

Let us now consider four hypothetical diets I, II, III and IV, and analyze them from the viewpoint of their possible effects on a certain diabetic patient weighing 50 kg. Let it be assumed that each diet is

⁹ Osborne and Mendel J Biol Chem **17** 366, 1914

completely digested, absorbed and catabolized, and that each is sufficient to cover the maintenance requirements of the body, so that we may in discussing them waive endogenous factors. Were these diets not sufficient to maintain the body, foodstuffs derived from the tissues would add themselves to the combination actually being utilized in the body and these would have to be known and allowed for. Also if some of the fat were deposited in the tissues this would have to be allowed for.

Looking at these diets simply as so many different combinations of carbohydrate, protein and fat, and going no further, they appear to be quite dissimilar. Who could certainly judge their relative effects on a patient? Diet I is a fair example of the old fashioned "rigid" diet, with almost no carbohydrate and the protein at 3 gm per kg of body weight. Diet II is a high carbohydrate, low protein diet. It is the kind of a combination that might possibly be given in a von Duering "rice cure," or a "cereal cure." This diet would permit the patient to enjoy 350 gm—almost a pint—of boiled rice or other cereal, or, he

TABLE 1—ELEMENTAL ANALYSIS OF FOUR HYPOTHETICAL DIETS

	I	II	III	IV
Carbohydrate	10	77	60	51
Protein	150	30	85	70
Fat	84	108	91	125

could have 134 gm white bread and butter—three slices—with each of his regular meals. Diet III appears to be intermediate between Diets I and II in all respects. It contains less carbohydrate than Diet I, but more than Diet II, and the fat is less than in Diet II and more than in Diet I. It contains nothing that is not contained in higher quantities by one of the other diets. Diet IV resembles Diet II, but might be suspected of having a higher caloric value because of its fat. We make a rapid estimation of calories and note that Diets I, II and III each represent 1,400, while Diet IV shows about 1,609 calories. On the basis of calories alone, if any of the diets is not to be tolerated, Diet IV would be the one to suspect. Now, if in each diet we add up the grams of carbohydrate plus 0.58 of the grams of protein, plus 0.1 of the grams of fat, we find that Diets I and II are alike in that each is capable of introducing 105 gm glucose into the metabolism. They are not only isocaloric, but will yield the same quantities of glucose. If we assume that the patient is capable of utilizing from 105 to 110 gm glucose per day he might very well tolerate either of these diets. Of the two, he would be less likely to tolerate Diet I. Coming to Diet III, and estimating the glucose equivalent G, we found it to be

118 This harmless looking isocaloric diet will surely cause glycosuria in the patient who is capable of utilizing only from 105 to 110 gm Diet IV, with 300 more calories than any of the other three, has the same glucose equivalent as Diets I and II and will probably be borne as well as either. The question arises in respect of Diet IV as to whether the high fat is permissible or in any sense objectionable. We estimate the higher fatty acid value of this diet F A by means of formula 2. Taking 0.46 the protein + 0.9 the fat, we find $32 + 113$ or 145 for F A. As stated, G is 104. Then the ratio $\frac{F A}{G} = 1.4$. As a general rule, we have found that acetone does not appear in the urine of an uncomplicated case of diabetes, or remain permanently if present when the ratio does not exceed this, provided, that the diet is completely absorbed, and catabolized and sufficient for maintenance, so that endogenous factors of food supply do not complicate the calculation. Accordingly, we say that the quantity and proportion of fat in Diet IV are probably not too high for complete utilization in the normal manner and that there is probably nothing objectionable about it.

This leads us to the problem of calculating the theoretical optimal diet.

ESTIMATION OF OPTIMAL DIETS

If C = carbohydrate, P = protein, F = fat, G = glucose and F A = higher fatty acids (plus ketogenic amino-acids expressed in terms of higher fatty acid), we may say—as shown above—that the quantity of glucose which any given combination of foods may introduce into the metabolism is expressed by the equation (1) $G = C + 0.58 P + 0.1 F$ and that the quantity of higher fatty acids (and equivalents) may be expressed as (2) $F A = 0.46 P + 0.9 F$. When the ratio $\frac{F A}{G}$ exceeds a certain value, ketonuria develops. Assuming that this ratio is 1.5, then $\frac{C + 0.58 P + 0.1 F}{0.46 P + 0.9 F} = 1.5$, when the ratio of fatty acids to glucose is as high as it may be without ketonuria. Simplifying this we obtain $F = 2 C + 0.54 P$, or, simply (3) $F = 2 C + \frac{P}{2}$. If it is agreed that the ratio F A / G shall not exceed 1.5 and that the relationships expressed in equations 1 and 2 are as given, then to estimate the optimal food combination or diet one may use equations 1 and 3. Given the quantity of glucose that the patient can utilize completely, assign this value to G in equation 1. Thus, if 100 gm is the highest quantity of glucose derived from all sources that the patient can utilize, $100 \text{ gm} = C + 0.58 P + 0.1 F$. In order to secure the maximal number of calories, the diet must clearly contain every possible gram of fat (at 9 calories per gram) that

the value of G and the relations expressed in 1 and 3 will permit, and consequently the lowest possible carbohydrate protein fraction (at 4 calories per gram) Also, as between carbohydrate and protein, the protein must be as low as possible and the carbohydrate as high as possible for 1 gm carbohydrate yielding 1 gm glucose and 4 calories provides for the normal oxidation of 1.5 gm of higher fatty acid On the other hand, 1 gm protein having the same caloric value as carbohydrate yields less glucose to support fat combustion and besides this yields acetone itself If the body weight of the patient be 50 kg and 1 gm protein per kg is selected as a conservative minimum, then P

becomes 50 gm and $F = 2C + \frac{P}{2}$ becomes $F = 2C + 25$ We

have already made $G = 100$ gm Now, the glucose yielded by the 50 gm protein will be 0.58×50 , or 29 gm, leaving $100 - 29$, or 71 gm, to be distributed between carbohydrate and fat In other words, $C + 0.1F = 71$ From this we obtain $F = 710 - 10C$

TABLE 2—SHOWING OPTIMAL FOOD COMBINATIONS WHEN $G = 100$ GM (IN THE EQUATION $G = C + 58 + 1F$), WHEN $FA = 15$, AND WHEN THE PROTEIN IS 0, 25, 50, 75 AND 100 GM (IF 0, 10, 15, AND 20 GM PER KG FOR A BODY WEIGHT OF 50 KG)

	P	C	F	Calories	Difference in Calories
(1)	0.000	83.333	166.666	1833.327	76.25 (2)-(1)
(2)	25.000	70.208	152.916	1757.076	76.26 (3)-(2)
(3)	50.000	57.083	139.166	1680.826	76.25 (4)-(3)
(4)	75.000	43.958	125.416	1604.576	76.25 (5)-(4)
(5)	100.000	30.833	111.666	1528.331	

No. 1 is hypothetical and could only be considered as the nonprotein fraction of a larger combination

But we also have from the above, $F = 2C + 25$ So $2C + 25 = 710 - 10C$, solving which $C = 57$ gm (57.08) Substituting this value for C in $F = 2C + 25$ we find $F = 139$ gm (139.16) Then, the optimal food combination that will fulfill the conditions and relations specified is carbohydrate, 57 gm, protein, 50 gm, fat, 139 gm = calories, 1,680 Proving this diet, we find the total glucose equivalent $G = 57.08 + (0.58 \times 50) + (0.1 \times 139.1) = 99.99$ as called for Also $FA = (0.46 \times 50) + (0.9 \times 139.1) = 150.24$ and $\frac{FA}{G} = 1.50$ as required

It is apparent, that any addition of any foodstuff to this diet will make G greater than 100 If, on the other hand, one added more fat—say 10 gm—and subtracted 1 gm carbohydrate, G would remain

100 and the calories would be increased by 86, but this would make $\frac{F \cdot A}{G}$ greater than 15. The effect of changing the protein can be seen by comparing the caloric value of a series of optimal food combinations with the protein rising from 0 to 2 gm per kg.

For each gram of glucose that can be utilized in the body there are 18 calories in the optimal food combination.

For each gram of protein in the food supply, subtract 3 calories from the optimal number.

For mental calculation, if one knows that a patient can actually utilize a certain number of grams of glucose, take this number times seventeen as the approximate number of calories that he will be capable of using at best without glycosuria or acetonuria if the diet is most favorably balanced. Then, knowing the patient's weight the severity or mildness of the situation becomes apparent.

REPORT OF CASE

To illustrate practical aspects of the principles discussed, the following case will be found of interest.

A Greek, aged 26, was admitted to the hospital as an emergency case. He was very weak, languid and emaciated, body weight 45 kg. There was a flush on the face, and the respirations were slightly increased when quiet in bed. The plasma carbon dioxide was 23.9 volumes per cent by Van Slyke. The breath smelled of acetone and the urine contained large quantities of sugar and acetone bodies. He had the symptoms and signs which are commonly taken to indicate a very severe case of diabetes.

First Stage. He was put to bed with artificial warmth and placed on a diet of 400 gm of 5 per cent vegetables with clear broth and plenty of water. But after four days, he still passed 32 gm sugar in the urine with acetone and 2.3 gm ammonia. His temperature on the fourth day ranged from 96.4 to 97.6 F, the pulse from 56 to 60, and he was so weak that it was not deemed safe to continue the effort to desugarize at that time. This ended the first stage of management.

Second Stage. He was then given for five days a diet containing 114 gm carbohydrate, 45 gm protein, 15 gm fat and 760 calories. G for this diet is 142 gm. During these five days he excreted on the average 85 gm glucose per day, so that he was disposing of about 67 gm glucose per day from the diet alone. His general condition improved and he was then desugarized by two days of complete fasting. Having remained sugar 'free' for one day, he was then given 400 gm vegetables of the group that contains not over 5 per cent of carbohydrate plus 1 liter of clear broth. This diet contained 12 gm carbohydrate, 14 gm protein and 104 calories, and G is 20 gm. But he promptly showed sugar in the urine and was again desugarized by one day of fasting. The patient had just manifested his ability actually to utilize at least 67 gm glucose, yet the mere feeding of a diet that could yield but 20 gm of glucose in the body induced glycosuria.

Third Stage. The urine was now free of abnormal quantities of sugar and acetone, and the patient was given a liter of broth and 200 gm of the fresh vegetables, which he bore without glycosuria. Then he received 400 gm greens, then 1 egg, and so on until the diet contained 314 calories, when the urine again showed sugar. Again, his condition was such that it was unsafe to continue

these tactics in the effort to maintain him in the sugar "free" state. Three weeks had passed and three successive attempts had failed, although at no time had the diet been capable of introducing into the body as much glucose as the patient had actually utilized on the 760 calories diet of the second stage.

Fourth Stage He was given a diet that contained 92 gm carbohydrate, 103 gm protein, 70 gm fat and 1,416 calories. G for this diet is 159. He was on this diet for two weeks and excreted on the average 50 gm glucose per day. Thus, he was actually utilizing about 109 gm glucose per day. His condition was much improved and he was then desugarized by two days of fasting.

Fifth Stage He was now in the sixth week of management, free of glycosuria and acetonuria after his third desugarization, and the attempt was again made to build up his diet gradually. But at 780 calories glycosuria recurred. A fourth desugarization by fasting was followed by another attempt, but glycosuria developed with a diet of 1,000 calories. He was desugarized a fifth time and again broke over at 1,000 calories. Nine weeks had elapsed. He was then established on a diet of 953 calories, with which he remained sugar "free." The diet now contained 50 gm carbohydrate, 67 gm protein and 56 gm fat, with $G = 88$. The plasma carbon dioxide was 60 volumes per cent. There was no abnormal quantity of acetone in the urine and the urinary sugar as measured by the method of Benedict, Osterberg and Neuwirth showed 454, 515, 486, 425, 494, and 400 mg, respectively, per day on six successive days (tenth week).

At 22 calories per kg, even with a subnormal basal rate, this was barely a maintenance diet and it was apparent that unless something better could be done, the outlook for the patient was hopeless.

Much has been written about the harmful influence of too much protein in the diabetic diet. Naunyn, von Noorden, Falta and others have emphasized the necessity of keeping the protein of the diet within limits. A new interest had been given this subject by the recent report of Newburgh and Marsh. We decided to test this idea to its limit. So the patient was placed as nearly as might be in a state of specific protein hunger.

Sixth Stage The diet was made up exclusively of rice and butter to contain 24 gm carbohydrate, 25 gm protein, 102 gm fat, and 1,024 calories. The calories were, therefore, a little higher than he had yet been able to receive without glycosuria. He was placed on this diet and remained sugar free with excretions of 305, 400, 370, and 392 mg respectively, on the four following days. There was no acetonuria. Now, the diet was rapidly increased, first by additions of rice alone, then by butter and rice together. In ten days it contained 84 gm carbohydrate, 115 gm protein, 162 gm fat and 1,837 calories. The urine at no time contained acetone and during these ten days had contained 308, 359, 384, 317, 545, 335, 439, 330, 486 and 333 mg respectively, of sugar. But the patient was not in nitrogen equilibrium. The urinary nitrogen was extremely low yet it alone was exceeding that of the diet by about 1 gm daily. So two eggs were added to the diet which made it contain carbohydrate 84 gm protein 25 gm (or 0.6 gm per kg), fat 174 gm and calories 2,000. On this (if the stools were neglected), the patient came into nitrogen balance. Thus, after nine weeks of trial, the ordinary practice of under-nutrition had failed to establish the patient in the nondiabetic state with more than 953 calories whereas after two weeks of the rice-butter diet the calories had more than doubled and the carbohydrate had been increased to 60 per cent. This seemed like a miracle. The 84 gm carbohydrate when served in the form of boiled rice made a mass of about 400 gm which was all that the patient would eat.

The contrast between the trays that now came to him and those that he had formerly had aroused many remarks in the ward. Surely, it might seem that there were merits in protein deprivation.

This is the type of result that astonished von Noorden when he first used his oatmeal cure. It is the type of result that has made the reputation of the von Duering rice "cure," the Mossé potato "cure," the Falta cereal "cures." However, if we examine this diet according to the equation and take the grams of carbohydrate plus 0.58 of the grams of protein, plus 0.1 of the gram of fat, we find that G is 116 gm, and it will be remembered that in the fifth stage the patient had shown his ability to utilize 109 gm glucose although at that time he had not remained sugar "free." He was not doing much more. The question arose as to what would have happened had the high protein diet of the fifth stage had a value for G less than 116.

Seventh Stage. A diet was composed to contain 28 gm carbohydrate, 118 gm protein (27 gm per kg instead of 0.6 gm), and 160 gm fat. Total calories, 2,024 (as against 2,000). G for this diet is 112. The patient was given this diet and remained sugar free and free of acetonuria. He remained on it for eight days when it was increased to find his tolerance limit. This was from 119 to 120 gm.

INTERPRETATION

Clearly, the merit of the rice-butter diet had not lain simply in its low content of protein. Four times as much protein had not been incompatible with a result just as good. In retrospect it is clear that the patient had shown from the second stage on the inherent ability to utilize a goodly amount of glucose. His actual tolerance was probably higher on the last day than it was on the seventh but as early as the fourth week he had burned almost as much glucose as he ever did later, so it can not be held that the final result was simply made possible by a rising tolerance. Notwithstanding his manifest ability to burn glucose, he had repeatedly shown glycosuria on very low diets that were incapable of introducing into his body as much glucose as he was able to burn, paradoxical as it may seem. This tends to encourage speculation. Shall we say that because of the general emaciation the starved kidneys had become abnormally permeable to sugar, that the "renal threshold for sugar" was lowered, introducing an element of "kidney diabetes"? Or, shall we evade the issue and say that in such cases the attempt should not be made to maintain the patient in the nondiabetic state, that such patients should be fed and allowed to run sugar until they are stronger, in spite of all that we know of the power of an excess of glucose to diminish tolerance. Shall we entertain the idea that a diabetic may burn more sugar if he is given an excess than he can if he is given just the quantity that he can utilize

without glycosuria? I should say no, these ideas are based on misconceptions of the food supply of the diabetic. *It was clear in this case from the events that followed, that the patient could be given every gram of glucose that he was capable of burning under any circumstances and still be kept from showing glycosuria, provided his food supply were suitably adjusted*

Earlier in the paper emphasis was laid on the necessity of conceiving the food supply of the diabetic patient as coming from the tissues as well as from the diet, especially when the diet falls below the maintenance requirements of the body. In the present case, we are dealing with such a situation. All of the low diets that aroused wonder because they induced glycosuria, even though incapable of introducing into the body as much glucose as the body could burn, were diets that failed to meet maintenance requirements. Consequently, when these diets were being used, the patient was drawing a greater or smaller part of his food supply from his tissues. It is not only necessary to consider the supply of food from the tissues, but to consider the kind and quantities of foods that will be supplied by the tissues of the particular case in hand. When a patient is thrown back on his own tissues for food, he can only draw on the materials that are there. As emphasized by the quotation from Lusk, when in fasting the body contains much fat, much fat burns and little protein, when there is little fat in the body, little fat burns and much protein, when there is no fat, protein alone burns. But whatever the composition of the body, something burns while life persists. This patient had very little fat in his body, and the quantity of glycogen was certainly negligible. Therefore, when this patient fasted, he must have produced much of his heat from protein. He weighed 43 kg. If in fasting he had produced only 15 calories per kg., this would have totaled 645 calories, an amount of energy contained in 161 gm. protein. If he catabolized this weight of protein, he would liberate 58 per cent of this weight, or 93 gm. glucose. Then, had he at this time the ability to burn 109 gm. glucose, the addition of 20 gm. from the diet would probably cause glycosuria. Unfortunately, in this case the basal metabolic rate and the urinary nitrogen were not actually determined during the fasting periods. But the literature contains many references to the "azoturia" of emaciated diabetics, and there can be no doubt that in such a case as this fasting induces a marked protein loss. Thus, fasting, in the case of a sufficiently emaciated patient, is the equivalent of a pure protein ration. In another case, with plenty of fat in the body, it is quite different as much tissue fat will burn and much less protein. It was pointed out earlier that the ingestion of

fat by a fasting individual who already has plenty of fat in his body has very little effect on the protein metabolism. If too much fat is ingested, it may increase acidosis and increase the protein loss but in the right quantities it simply replaces tissue fat. But, if the body fat is already depleted and if, as a result of this the protein breakdown has already become excessive, then the ingestion of fat will retard the prevailing protein breakdown and reduce it to a lower level. Thus in the emaciated individual, the ingestion of fat spares protein. In this diabetic patient, in this particular state, the feeding of enough fat should reduce the protein catabolism and thereby reduce the quantity of glucose thrown into circulation. When he was given the rice-butter diet he received at once 102 gm fat for 918 calories, almost enough to replace his minimal fasting caloric requirements, and this put a rapid quietus on the excessive protein breakdown. The quantity of glucose from protein having thus been cut down, there was then opportunity to introduce more carbohydrate in the diet which further suppressed the protein breakdown. *The principal reason why this diet operated so well was because of its content of fat and because the body was so depleted in fat at the time when it was given. The patient was in a state of extreme fat starvation.* When in the sixth stage of management the patient did equally well on a low carbohydrate high protein diet this was because the combined carbohydrate and protein of that diet introduced into the body no more glucose than had formerly come from the combined carbohydrate and protein of the rice-butter-egg combination, and because this diet also contained enough fat to prevent endogenous factors of food supply from coming into action. Thus, it will be seen how the administration of a diet in excess of maintenance requirements may under certain conditions actually reduce the quantity of glucose in circulation in the body. Were the fat dropped out of these last two diets, more glucose would be thrown into metabolism by the increased breakdown of tissue protein induced by the lowering of the diet. This, I take it, is the explanation of a certain type of glycosuria which develops with prolonged undernutrition. I have seen a patient with diabetes similar to this one who could not be desugarized at all by fasting, but who cleared promptly when enough fat had been added to the diet with no other changes. This man, in fasting, catabolized so much protein that his protein sugar overtaxed this tolerance limit.

ACUTE YELLOW ATROPHY OF THE LIVER*

MAX KAHN M.D., PH.D., AND JOSEPH BARSKY, M.D.
NEW YORK

The pathology of acute yellow atrophy of the liver, idiopathic in its etiology, was first described by Rokitansky, who regarded the process as one of "bilious liquefaction." According to him, an excess of the elements of bile is formed in the blood of the portal vein which prevades the entire vascular apparatus of the liver, and causes destruction of the hepatic glandular structure by liquefaction. This view, which is obsolete now, was partially concurred in by Henoch and by Von Dusch. Henoch thought that the atrophy was due to a polycholia with consequent distention of the biliary capillaries and resultant compression of the blood vessels which ultimately interferes with the hepatic nutrition. Von Dusch ascribed the disease to a paralysis of the bile ducts and lymph vessels which gives rise to an infiltration of the organ with bile, and finally to a cellular destruction.

Bright was the first to attribute acute atrophy of the liver to a diffuse inflammation of the gland. To this theory, Engle, Wedl and Bamberger subscribed, and they have explained the destruction of the cells by a fatty degeneration arising from an acute exudation process.

Since Frerichs¹ detected leucin and tyrosin crystals in the urine of patients suffering from acute yellow atrophy of the liver, several investigators have studied the chemical changes which the hepatic tissue undergoes in this disease, and with increasingly improved analytical processes, have been able to give us more and more exact data as to the change in composition induced by this disease.

The study of the chemistry of the liver of acute yellow atrophy is especially interesting since it is the most striking example of intravital autolysis that occurs in human pathology. As Wells² points out, it excels the autolysis of pneumonia in interest since in the latter it is merely the exudate that is digested, whereas in the acute atrophy it is the liver itself that is destroyed. Sometimes as much as three quarters of the entire hepatic parenchyma is autolyzed within several days.

The analyses of Sootbeer,³ Beebe⁴ and Heffter⁵ were made to determine certain individual fractions in the atrophied liver. Soot-

* From the Department of Laboratories, Beth Israel Hospital.

1 Frerichs Diseases of the Liver **1** 160, 1879

2 Wells, H. G. J Biol Chem **5** 129, 1908, Arch Int Med **1** 589 (July) 1908, J Exper M **9** 627, 1907, Wells and Bassoe J A M A **44** 685 (March 4) 1905

3 Sootbeer Arch f exper Path u Pharmacol **50** 294, 1902

4 Beebe Am J Physiol **14** 237, 1904

5 Heffter Arch f exper Path u Pharmacol **28** 97, 1891

beer failed to find any amino-acids in the liver of acute yellow atrophy. Beebe found a normal or slightly increased pentose content in such livers. Heffter found decreased lecithin content in his two cases, but Wells doubts whether these two cases were instances of true acute yellow atrophy.

Perls and Von Starck⁶ reported that the liver of acute yellow atrophy contains a greatly increased amount of water, whereas the fat percentage was normal. A. E. Taylor⁷ also found an increased water content of these atrophic livers. He found no free arginin, histidin or lysin by the Kossel and Kutscher method. By Fischer's ester process he obtained 0.35 gm. leucin and 0.612 gm. aspartic acid, but he could not isolate any tyrosin.

H. G. Wells, in an exhaustive study of the chemistry of the liver of a young man who died of typical "idiopathic" acute yellow atrophy of the liver after an illness of six weeks, isolated and identified the following amino-acids: Histidin, lysin, tyrosin, leucin, glycocoll, alanin, prolin, glutamic acid, aspartic acid. These were found free in extracts of the liver, and presumably represent products of the autolysis of liver cells, although the amount of soluble nonprotein nitrogen present in the extracts was so large as to suggest that there must be some other source for these substances.

Small quantities of free proteoses and peptones, and of xanthin and hypoxanthin, were also found in the extracts.

In the insoluble proteins of the liver, the proportion of diamino-acids was decreased slightly as compared with normal livers. The proportion of protein phosphorus was increased, probably because of active regenerative proliferation, while the sulphur was normal in amount. Iron was increased because of the large quantity of blood in the liver and the hematogenous pigmentation of the liver cells.

Gelatinous material was increased both absolutely and relatively, because of the loss of parenchyma and the proliferation of the stroma.

The proportion of water to solids was much increased, there having been a loss of two-thirds of the entire parenchymatous elements of the liver. The amount of fat, lecithin and cholesterol was not far from that which is normal for the liver.

It is apparent that the total amount of lecithin in acute yellow atrophy is very greatly reduced, not only as to the actual amount present, but also in its relation to the other constituents of the liver. It would seem that although the liver has lost even as great a proportion of its fatty constituents as of its proteins, it has suffered an even greater loss in its lecithin. The significance of this observation cannot

⁶ Perls and Von Starck quoted by Quincke. *Spec. Path. u. Ther.* **18**: 297, 1899.

⁷ Taylor, A. E. *Univ. California Publications Pathology* **1**: 43, 1904, *Ztschr. f. phys. Chem.* **34**: 580, 1902, *J. M. Research* **8**: 424, 1902.

be estimated until we have more figures on the variations in the lecithins of the liver and other organs in health and disease. It is interesting to note, however, that in the liver showing severe chloroform necrosis, with considerable fatty change, there has also been a decrease in lecithin, although not so marked as in the acute yellow atrophy liver.

The cholestrin has not been so greatly reduced, for this liver shows about the same proportions, and nearly as large a total amount of lecithin, as the controls. The reduction in amount of neutral fats and lecithin causes it to form an unusually large proportion of the ether extract.

In a case of hepatic necrosis due to chloroform, Taylor⁷ found in the liver four grams leucin, 2.2 grams tyrosin, and 2.3 gm arginin nitrate.

Wells who studied the chemistry of the liver in chloroform necrosis reported a rapid autolysis of the liver cells, resulting in a loss of as much as one-third or more of the solids in three or four days, and indicated chemically by the presence of free amino-acids, purins, proteoses, peptones and polypeptids in the liver. Several of the amino-acids were present in quantities large enough to permit of their isolation and identification. Despite the loss of nearly all the nuclear structures of the liver the amount of insoluble phosphorus was found increased in the specimen examined, without alteration in the amount of insoluble sulphur. The distribution of the nitrogen as mono- and diamino-acids in the insoluble coagulated liver proteins is not different from that of proteins of the normal liver. There is a moderate degree of fatty metamorphosis, the microscopic and chemical findings corresponding in this respect, this increase of ether-extractable material being due to infiltration of simple fats while there is a slight decrease in the lecithin and no alteration in the cholestrin. There is less replacement of proteins by water and more fatty infiltration than in acute yellow atrophy.

Stadie and Van Slyke⁸ studied a case of acute yellow atrophy in a patient who died within a week of the first onset of symptoms. The urine showed a high ammonia nitrogen (from 12 to 17 per cent of the total nitrogen), high amino-nitrogen (4.1, 16.0 and 13.3 per cent of the total nitrogen on last three days), and low urea nitrogen (from 47 to 52 per cent of total nitrogen), and contained tyrosin. The blood contained from 8.8 to 15.9 mg urea nitrogen, and from 14 to 26.3 mg amino-nitrogen per 100 cc. Since the patient had no food, the amino-nitrogen must have been derived from the tissues. On the last two days before death, there was a high excretion of titratable acid and ammonia and one the day before death, the plasma carbonate fell slightly below normal (49 per cent carbon dioxide) but these changes

⁸ Stadie and Van Slyke. *Arch. Int. Med.* **25** 693 (May) 1920.

were too slight to be of great significance. The liver weighed 1,000 gm (supposed normal 1,800 gm) and contained 71.7 per cent water and 13.5 per cent fat. Per 100 gm liver, there were 2.38 gm nitrogen and 0.315 gm nonprotein nitrogen (heat coagulation and kaolin employed to remove protein). Of this 4.76 per cent was urea, 10.95 per cent ammonia, 42.5 per cent amino-nitrogen, 22.8 per cent peptid amino-nitrogen, 4.54 per cent creatin, 1.05 per cent creatinin and 13.4 per cent undetermined. Attention is called to the fact that neither during resolution in pneumonia nor after intoxication with proteose or by intestinal obstruction, in all of which there is very rapid autolysis, is there an increased excretion of amino-acids, nor does this occur in hepatic disorders, except when the destruction of liver cells is almost complete. "These observations support the view that in the deamination of amino-acids and the synthesis of urea the liver bears a part which cannot be entirely assumed by the rest of the body."

We wish to place on record here studies of two cases of acute yellow atrophy of the liver. One of these (Case 1) was studied in the Western Pennsylvania Hospital of Pittsburgh, the pathologic diagnosis having been made by Dr. Vernon Lee Andrews, the pathologist. The second case was studied in collaboration with Dr. Joseph Barsky, the pathologic description was made by Dr. Eli Moschcowitz. In the second case, certain general chemical studies were also made. The history of Case 2 will be found at the end of this paper.

The chemical analysis of the livers reported in this paper may be classified as follows:

1. Water content and elemental composition
2. Study of the lipid fractions—lecithin, cholesterol, soaps, fatty acids and fats, etc.
3. Study of the nitrogen fractions—protein and amino-acids

Water Content—The liver of acute yellow atrophy is characterized by a high water content, therein differing from the necrosis of the liver induced by phosphorus poisoning, chloroform poisoning or the fatty degenerated liver.

In liver 1, the water content was 82.75 per cent, dry matter, 17.25 per cent; in liver 2, the water content was 75.36 per cent, solids, 24.64 per cent. In other cases of liver atrophy, the water percentages shown in Table 1 were found.

In acute yellow atrophy, the moisture of the liver is often much increased at the expense of the proteins which have been autolyzed. In Case 2, however, the water content was normal.

The ash in the liver of acute yellow atrophy is markedly increased. We found the ash to be 2.40 per cent in case 1 and 2.72 per cent in case 2, whereas in the normal livers of human beings, we found from

1 178 to 1 347 per cent Of the ash the soluble salts are the ones that are mostly increased whereas the water insoluble mineral matter is reduced in quantity The water soluble fraction of the ash was 95 77 per cent in Case 1 and 92 35 per cent in Case 2, and the insoluble mineral matter was 4 23 per cent of the ash in Case 1 and 7 65 per cent in Case 2 In normal livers we found the soluble salts to be from 72 4 to 81 7 per cent of the ash, and the insoluble salts from 27 6 to 18 3 per cent of the ash

TABLE 1—WATER PERCENTAGES OF LIVERS OF CASES OF ACUTE YELLOW ATROPHY OF THE LIVER

Normal liver (Quincke)	76 1
Normal liver (Wells)	77 6
Acute atrophy (Perls)	81 6
Acute atrophy (Perls)	76 9
Acute atrophy (V Starck)	80 5
Acute atrophy (Taylor)	85 8
Acute atrophy (Wakeman)	79 3
Acute atrophy (Wells)	83 8
Acute atrophy (Voegtlin)	78 0
Acute atrophy (Kahn)	82 75, 75 36
Acute atrophy (Stadie and Van Slyke)	71 7
Phosphorus poisoning (V Starck)	60 0
Fatty degeneration (V Starck)	64 0
Chloroform necrosis (Wells)	72 4

TABLE 2—PERCENTAGE COMPOSITION OF NORMAL LIVER SUBSTANCE

	Parts per 1,000
Average dry substance	160 26
Average sodium chlorid	2 806
Average sodium	0 455
Average potassium	1 718
Average calcium	0 124
Average magnesium	0 143
Average iron	0 614
Average phosphorus	2 551
Average sulphur	1 775
Average phosphate	3 291
Average sulphur trioxid	0 268

TABLE 3—MINERAL CONSTITUENTS IN ASH OF LIVER TISSUE

	Adult	Child
Potassium	25 23	34 72
Sodium	14 51	11 27
Magnesium	0 20	0 07
Calcium	3 61	0 33
Chlorin	2 58	4 21
Phosphorus pentoxid	50 18	42 75
Sulphur trioxid	0 92	0 91
Silicon dioxid	0 27	0 18
Iron oxid	2 74	

Dennstedt and Rumpf⁹ obtained percentages composition of normal liver substance as shown in Table 2

In the ash of liver tissue Oidtman¹⁰ found the mineral constituents shown in Table 3

⁹ Dennstadt and Rumpf *Ztschr f klin Med* 58 84, 1905

¹⁰ Oidtman See Forbes and Keith, *Phosphorus Metabolism*, 1915, p 105

Von Moraczewski¹¹ analyzed the organs of subjects who had died from various diseases. In the livers of such patients he found the percentage composition shown in Table 4, with which it is well to contrast our findings in the cases of acute yellow atrophy.

The analysis of the liver of acute yellow atrophy, so far as its inorganic analyses go, is shown in Table 5.

Contrasting our results with Oidtman's, we find that in acute yellow atrophy there is a marked increase in the content of the sodium salts, so much so, that they outbalance the potassium salts (Cases 1 and 2). The chlorine, sulphur and magnesium contents are increased, whereas the calcium, iron and silica are reduced (Case 1).

TABLE 4—PERCENTAGE COMPOSITION OF LIVERS OF PERSONS DEAD FROM VARIOUS DISEASES

Disease	Dry Matter	Nitrogen in Fresh Substance	Chlorine in Fresh Substance	Phosphorus in Fresh Substance	Phosphorus in Dry Substance	Calcium in Fresh Substance
Pneumonia F	34.1	4.071	0.092	0.189	0.55	0.001
Pneumonia M	11.7	1.584	0.271	0.932	7.99	0.004
Carcinoma, starvation	17.2	2.701	0.191	0.216	1.25	0.017
Carcinoma, starvation	28.6	2.022	0.184	0.180	0.62	0.001
Carcinoma, anemia F	38.6	2.712	0.174	0.199	0.51	0.004
Carcinoma, anemia M	17.4	2.561	0.153	0.237	1.35	0.001
Pernicious anemia F	9.1	2.285	0.216	0.154	1.69	0.003
Pernicious anemia M	17.2	2.565	0.125	0.217	1.25	0.017
Death by bleeding M	4.3	2.945	0.209	0.099	2.30	0.012
Normal state			0.027	0.388		0.028

TABLE 5—INORGANIC ANALYSES OF LIVER OF ACUTE YELLOW ATROPHY

	1 Per Cent	2 Per Cent
Moisture	82.750	75.36
Dry matter	17.250	24.64
Organic matter	14.841	21.92
Ash	2.409	2.72
Soluble part of ash	2.405	2.51
Insoluble part of ash	0.004	0.21
Sodium	27.34 of ash	24.86 of ash
Potassium	27.17 of ash	23.19 of ash
Calcium	2.19 of ash	
Magnesium	0.82 of ash	
Iron	0.43 of ash	
Phosphorus	41.52 of ash	
Sulphur	1.24 of ash	
Chlorine	5.19 of ash	5.38 of ash
Silica	0.09 of ash	

Wells found in the alcohol insoluble residue of the liver tissue of a case of acute yellow atrophy the following average percentages:

Ash	1.9	Phosphorus	0.50
Sulphur	0.82	Iron	1.22

The Lipins—The total fat in the liver of acute yellow atrophy was found to be 2.17 per cent in Case 1 and 8.32 per cent in Case 2. Quincke found 3.0 per cent and Wells 5.0 per cent fat in normal livers. In acute yellow atrophy, the liver contains a smaller proportion

¹¹ Von Moraczewski, Ztschr. f. phys. Chem. **23**, 385, 1896.

of fat, according to the analyses of Taylor and of Wells. Other observers, have, however, reported increased fat content of the liver that has undergone acute yellow atrophy. In the fatty degeneration of the liver due to phosphorus poisoning, chloroform necrosis, etc., high liver fat contents have been found. Table 6 gives the comparison of the fat analysis of such livers by different authors.

It seems, therefore, that the fat content is not constantly affected in this condition.

The Lipin Partition—The lecithin fraction was found to be much decreased in one case, in this regard confirming a similar finding of Wells. The percentage of lecithin in the fresh liver tissue of acute atrophy was 0.32 per cent, whereas in normal livers, Wells found from 1.4 to 1.6 per cent, being more in the anemic and less in the congested liver. In the liver of acute atrophy, Wells found 0.45 per cent lecithin. In the second case, the lecithin was 0.87 per cent.

TABLE 6—FAT ANALYSIS OF LIVERS

	Per Cent
Normal liver (Quinke)	3.0
Normal liver (Wells)	5.0
Acute atrophy (Perls)	8.7
Acute atrophy (Perls)	7.6
Acute atrophy (v. Starck)	4.2
Acute atrophy (Taylor)	2.0
Acute atrophy (Wells)	2.5
Acute atrophy (Voegtlin)	26.6
Acute atrophy (Stadie and Van Slyke)	13.5
Phosphorus poisoning (v. Starck)	29.8
Chloroform necrosis (Wells)	8.8
Fatty degeneration (v. Starck)	25.0
Acute atrophy (Kahn) Case 1	2.17
Case 2	8.32

The cholesterol content of the acutely atrophied liver of Case 1, was not decreased at all. It was found to be 0.41 per cent of the total liver substance. In normal cases, Wells found from 0.26 to 0.37 per cent of cholesterol, and in acute atrophy 0.3 per cent cholesterol. In Case 2, the cholesterol amounted to 0.35 per cent. It seems, therefore, that in the autolytic process which attacked the liver, the lecithin had been destroyed, whereas the cholesterol fraction remained unaffected.

In Case 1, phosphatids were very much reduced. In the dry portion of the liver, we found 7.63 per cent phosphatids, which is a decrease from the normal by about 50 per cent. For example, in the dried portion of normal liver, Grimew found 15.9 per cent phosphatids. This reduction in the phosphatids is also present in tuberculous inflammation of the liver, in which case Grimew obtained a phosphatid content of 9.5 per cent of the dry matter of the liver.

Sulphatids are present normally in the liver, according to our analysis, to the extent of 0.8017 per cent of the total fresh substance. In the liver of acute yellow atrophy, the sulphatids are reduced to

0.204 per cent The sulphatids were determined by the method of Koch, slightly modified The autolytic process, it would seem, breaks down these sulphatids and liberates the sulphate ion which unites with some of the metallic elements, thus increasing the sulphur content of the ash

Study of the Nitrogen Fractions—Wells found in his analysis of the proteins of the liver in acute yellow atrophy nitrogen fractions as shown in Table 7 (He used Hansmann's method)

TABLE 7—NITROGEN FRACTIONS IN LIVER

	Acute Atrophy	Normal (Anemie)	Normal Congested	Chloroform Necrosis
Amid nitrogen	3.5	3.7	4.8	3.9
Humus nitrogen	3.6	3.4	4.9	5.7
Diamino nitrogen	26.2	32.8	30.0	30.0
Monamino nitrogen	64.8	60.3	60.2	60.3

According to Wells, "There seems to be present here as Wakeman found in his dog's livers, a decrease in the diamino nitrogen, although this is not so striking as in Wakeman's material¹² Possibly the slighter decrease observed in the acute yellow atrophy liver depends in part upon an increase in the purins present on account of regenerative cell multiplication, for in the Hausmann method of determining nitrogen distribution, the purins are partly precipitated with diamino compounds It was impossible to determine the relative proportion after the histidin had been separated However, the proportion of nitrogen present in the form of histidin (0.54 gm) to that present as arginin and lysin (0.94 gm) is larger than the normal proportion, and suggests that either the arginin or the lysin, or both, were decreased much below the normal"

From a liver of acute yellow atrophy Wells isolated various amino-acids, as shown in Table 8

TABLE 8—AMINO-ACIDS IN LIVER OF ACUTE YELLOW ATROPHY

	Grams
Histidin	0.64
Lysin	1.04
Tyrosin	0.70
Leucin	4.16
Glycocoll	0.20
Alanin	0.30
Prolin	0.35
Glutaminic acid	1.00
Aspartic acid	0.28
Total	8.67

Wells remarks, "The quantities given above indicate nothing as to the actual amounts of the free amino-acids that were present in the liver, as will be appreciated by anyone who has worked with these

¹² Wakeman J Exper M 7 293, 1905

substances, for our analytic methods are so imperfect that the quantities obtained represent merely minimal figures, and account for only such quantities of each amino-acid as I could obtain in sufficient purity for positive identification. How small a part of the total quantity of amino-acids that was really present in the liver is represented by the isolated and identified amino-acids, is shown by the fact that the 8.67 gm of amino-acids obtained accounts for but about 1.5 gm of nitrogen that was present in the liver extracts from which the amino-acids were obtained. In the entire material used there was but about 64 gm of protein, which would contain about 10 gm of nitrogen, therefore, it is noteworthy that nearly one-third of the nitrogen of the liver was in a water-soluble, nonprotein form."

Leucin, tyrosin, lysin, arginin and aspartic acid have been found free in the liver or blood of persons dying with acute yellow atrophy or similar conditions.

Abderhalden and Barker¹³ found glycocoll in the urine of dogs poisoned by phosphorus. Wohlgemuth¹⁴ has found glycocoll, alanin and arginin in the urine of human beings poisoned by phosphorus. Wells found prolin and glutaminic acid free in the liver of acute yellow atrophy, and he states that "there is no evident reason why all the amino-acids that have been isolated from proteins by Fischer and others might not be found in diseased tissue in which autolysis has occurred."

13 Abderhalden and Barker. *Ztschr f phys Chem* **42** 524, 1904.

14 Wohlgemuth. *Ztschr f phys Chem* **44** 74, 1905, *Biochem Ztschr* **1**. 161, 1906.

The following references also may be consulted.

Balthazard. *Compt rend Soc Biol* **53** 922, 1901.

Fiessinger. *Compt rend Soc Biol* **60** 870, 1906.

Guleke. *Arch f klin Chir* **83** 602, 1907.

Guthrie. *Lancet* **2** 1542, 1906.

Neuberg and Richter. *Deutsch med Wchnschr* **30** 499, 1904.

Orlow. *Biochem Zentralbl* **5** 937, 1907.

FOOD ALLERGY AS A CAUSE OF ABDOMINAL PAIN

WILLIAM W DUKE, M D
KANSAS CITY, MO

Severe abdominal pain is a symptom which is never taken lightly by a careful physician. It often indicates a severe illness, in fact, often indicates an emergency. However, abdominal pain which may simulate in some respects the pain caused by serious abdominal disease is occasionally the result of hypersensitiveness to a food.

It has been known for a number of years that hypersensitiveness to foods may give rise to bronchial asthma and a condition which simulates hay fever, to urticaria, angioneurotic edema, purpura, eczema and other dermatoses, to dyspepsia, gastro-intestinal upsets associated with vomiting, diarrhea, gripping pains in the abdomen and mucous colitis, to an interesting syndrome of symptoms known as Henoch's purpura, and that with the latter conditions a patient may have severe abdominal pain. Abdominal pain occasionally occurs alone, however, or rather, it is occasionally the sole striking symptom of hypersensitiveness to a food. In this case, the real cause of pain may not be apparent, and an error in diagnosis can easily be made. It is this type of illness that I wish to discuss.

The condition is well illustrated by the case reports. The cases have been chosen from a larger number to illustrate typical histories of patients who have abdominal pain as the outstanding symptom of hypersensitiveness to a food. For this reason only such details as pertain to this subject will be related.

HISTORY

The phenomenon of sensitiveness or hypersensitiveness to alien matter has been discussed in its relationship to infection and immunity for many years. A cutaneous reaction obtained by applying smallpox virus to a scarified area on the skin of an immune individual was described in detail by Jenner as early as 1789. Magendie in 1839, and Flexner in 1894, apparently observed anaphylaxis in animals after inoculation with foreign material. Their observations were overlooked, however, and the subject did not attract much attention in medical literature until after the publications of Richet, in 1902. While experimenting with a toxin extracted from actinae, Richet found that animals inoculated with this poison became unusually sensitive to it—in fact so sensitive that a second dose, too small to be harmful to normal

animals, was sufficient to cause certain violent symptoms in animals which had previously been inoculated. This peculiar condition Richet termed anaphylaxis.

Following Richet's work, a number of valuable pioneer contributions were made by other observers working independently of Richet or along different lines, namely Arthus, in 1903, Pirquet and Schick, in 1903, Otto, investigating an observation made by Theobald Smith, Rosenow and Anderson, Vaughan, Besredka, Gay and Southard, and others. A number of remarkable facts were disclosed, the more important of which may be summarized as follows. Certain animals (particularly the guinea-pig) when inoculated with an alien albumin may become sensitive to that particular albumin. After being sensitized, future inoculations, instead of being practically harmless, are likely to cause violent symptoms of anaphylaxis which frequently result in the death of the animal. It was discovered that sensitization occurs only after an incubation period of several weeks or months has elapsed, that sensitiveness once established is likely to persist for years, even throughout the life of an animal, that the condition can be transferred passively to other animals by an inoculation of serum from a sensitized animal, that it can be transmitted from mother to offspring, and that sensitiveness may be removed quickly and completely by inoculation with sublethal doses of the albumin to which the animal is sensitive.

It was discovered that sensitiveness is remarkably specific for the type of proteid used in producing the sensitive state. For example, animals inoculated with milk might become remarkably sensitive to further inoculations with milk but not sensitive to serum—even serum of the same species. Likewise, animals sensitized against egg albumin might become sensitive to the particular egg albumin used in making the inoculation but not sensitive to other egg albumins nor to the serum of the same animals. Animals would be more sensitive to egg albumin of closely related species than to the serum of the same animal.

In 1910, Meltzer made a unique contribution to our knowledge of the subject. Comparing the bronchial constriction of asthma with that found in animals dying in anaphylactic shock, he suggested that real bronchial asthma was nothing more nor less than a phenomenon of anaphylaxis. This interesting suggestion attracted a great deal of attention, and since then a number of additional conditions have been attributed to a disturbed function caused by a remarkable hypersensitive state in humans which resembles anaphylaxis in many respects, but which seems to be fundamentally a different phenomenon. This state is described by more recent writers under the broader term "allergy."

The more interesting symptoms of allergy mentioned by Coca, Cook and others are, hay-fever, bronchial asthma, chronic joint disease,

urticaria, angioneurotic edema, certain eczemas, certain gastro-intestinal conditions with symptoms such as abdominal pain, indigestion, vomiting and diarrhea and a symptom complex which is generally described under the term Henoch's purpura

Whereas anaphylaxis and allergy have features and symptoms in common, they seem to be fundamentally different phenomena. This fact has been brought out especially by Coca, who mentions the following striking differences between the two conditions

First—The exciting agent of anaphylaxis is always antigenic in character. The exciting agent of allergy may or may not be an antigenic substance. (For example, it may be a drug such as arsphenamin or acetylsalicylic acid.)

Second—Although anaphylaxis may be transmitted from mother to offspring, it is not inheritable in the true sense of the word. It is always primarily an artificial condition induced by the introduction of antigenic substance into the body of some susceptible animal.

Allergy, on the other hand, is always based primarily on a natural inherited makeup. The sensitiveness classed as allergy does not in every instance appear to depend on previous contact with an exciting substance. An individual, for example, may have a violent allergic reaction when he comes in contact with the exciting substance apparently for the first time.

Third—The phenomenon of desensitization which can quickly and invariably be brought about in animals with anaphylactic sensitiveness is entirely wanting in allergy. The state of reduced sensitiveness observed clinically after treatment of allergy by hypodermic injections of the exciting agent is never complete nor is it comparable with the above.

For a complete discussion of these many interesting phases of this subject the writings of Coca, Cook, Walker, Vaughan, Besredka, Wells and others should be consulted.

REPORT OF CASES

CASE 1—Male, aged 32, with family and past history negative for hay-fever, asthma and other symptoms of allergy, had a severe illness at the age of 2, due to bee stings. He remembers that since earliest childhood he has invariably experienced a severe nonradiating gripping pain in the epigastrium, associated with nausea and vomiting, every time he has eaten honey. He has frequently tried to eat honey, but found that it invariably caused a severe attack of abdominal pain. The pain would start about fifteen minutes after the ingestion of honey, and last about three hours.

Previous Illnesses—The patient has always been well otherwise. He has been free from other digestive disturbance and has never noticed other symptoms of allergy either during the attacks described nor between attacks.

Physical Examination—Negative, except for chronic ethmoiditis. A cutaneous test made by applying honey to a scarified area on the skin gave a typical urticarial wheal about 2 cm in diameter, which appeared in less than five minutes and disappeared after about one-half hour.

The patient was unwilling to eat honey as a clinical test or to allow a small amount to be injected subcutaneous.

CASE 2—Patient, male, aged 37, with negative past history, while out camping about ten years ago missed two meals and then ate an entire cake of honey on an empty stomach without other food. He experienced no discomfort at the time. Two months afterward he observed that whenever he ate honey, even in small quantities, he invariably experienced a steady, severe pain in the epigastrium, which lasted several hours. This condition has persisted to the present time. The pain can be brought on at will by the eating of a small amount of honey.

Previous Illnesses—The patient gives no further history of dyspepsia, pain or symptoms of allergy, except those mentioned. His entire history and physical examination is negative, except as mentioned.

On ingestion of one teaspoonful of honey, as a clinical test he had epigastric pain which lasted one-half hour.

A cutaneous test made by applying honey to a small scarified area on the skin gave rise in four minutes to a typical itching wheal 0.5 cm in diameter, which persisted for one and one-half hours.

CASE 3—Male, aged 25, having a family and past history negative for hay-fever, asthma, dermatoses and other symptoms of allergy, ate shad roe from two to five times a week at lunch for a period of several months. After removing to another locality, he did not eat shad roe for a period of about two years. He then became subject to occasional attacks of severe nonradiating, steady pain in the epigastrium which would develop about fifteen minutes after a meal, and last from three to five hours. Occasionally, the pain was associated with nausea and vomiting. The pain was so severe as to completely incapacitate him for mental or physical work. Several attacks were followed by tenderness over the appendix, which lasted for about a week. The case was finally diagnosed recurrent appendicitis and the appendix was removed. A few adhesions about the appendix were found, together with several concretions in its lumen. Similar attacks of epigastric pain occurred after the appendix was removed. They differed from the former attacks only in the fact that they were not followed by tenderness in the right iliac fossa. Finally, it was discovered that the attacks occurred invariably after the eating of shad roe and that if the shad roe was eliminated from the diet entirely no such attacks occurred. The patient had not at any time had hives, asthma or other symptoms of allergy.

This sensitiveness has now persisted for more than ten years, but is not so marked as it was originally. The ingestion of shad roe at the present time causes discomfort in the abdomen and nausea, but not severe pain.

Physical Examination—Disclosed nothing of interest in this connection, except a moderate grade of ptosis.

An intracutaneous injection (made recently) with an aqueous extract of shad roe gave rise to a cutaneous reaction about 0.5 cm in diameter.

CASE 4—Patient, female, aged 45. Her father had autumnal pollen asthma until his death several years ago. Her brother had summer and autumnal hay-fever and also eczema on the hands until he was forced to leave this climate.

Previous Illnesses—The patient gives no history of hay-fever, asthma, eczema nor other symptoms of allergy. Eight years ago abscess of the appendix formed and was drained. Five years ago she had an infected gallbladder, which was removed. One year after this, for the first time in her life, she began having indigestion, the outspoken symptom of which was a severe steady pain in the epigastrium, which would come on every day about one-half hour after eating, and last from one to three hours. It was often associated with vomiting. She found by experience that the pain was relieved to a certain extent by lavage. The patient had been dieted and treated without relief for several months before coming under my observation. The history was otherwise negative or of no interest in this connection.

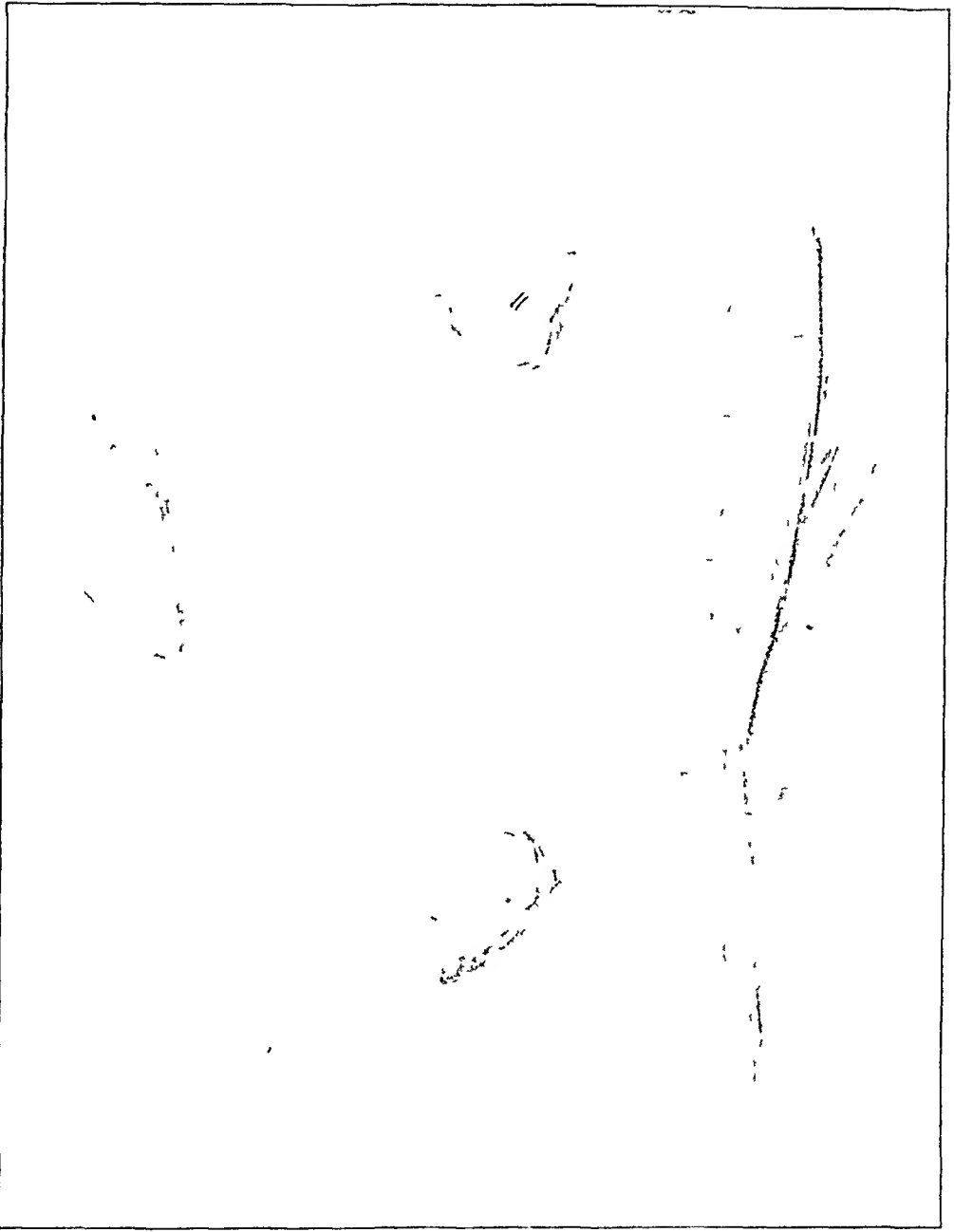


Fig 1—Positive cutaneous and intracutaneous tests The two tests were made at the same time by applying honey (above) to a scarified area on the skin, and (below) by inoculating 0.02 mg of honey intradermally Both reactions appeared in less than four minutes, itched intensely and disappeared after about one and one-half hours

Physical Examination—On examination was found a slight grade of oral sepsis, slight general abdominal tenderness, high grade ptosis, adhesions about the pyloric end of the stomach very evident by roentgen-ray examination and a bulb deformity, due evidently to adhesions. Examination was otherwise negative or of no interest.

Careful dietary and intracutaneous tests proved the patient to be sensitive to lactalbumin, beef proteid and egg white. Tests made by applying the above proteins in dry powdered form to a scarified area on the skin were negative. However, intracutaneous injection of the proteins mentioned gave rise in a few moments to typical wheals about 0.5 cm in diameter. Subcutaneous inoculations with small amounts of milk, egg white and beef serum, used subsequently in therapy, frequently gave rise to attacks of abdominal pain and vomiting similar to those of which the patient complained originally. The ingestion of a small amount of beef, milk and egg white, taken as a clinical test, in each instance gave rise to attacks of abdominal pain and vomiting, which came on within less than one-half hour and lasted from one to three hours. Other foods tested in this way caused little or no distress when she was on a diet free of egg, milk and beef proteins.

The patient was treated with subcutaneous inoculations of beef, milk and egg white (increasing from 0.001 mg to 1 mg) over a period of two months. She did not at any time during this treatment have hives, asthma, or other cutaneous or respiratory symptoms of allergy. A number of reactions, however, confined her to bed several hours with abdominal pain, vomiting and general malaise.

At the end of two months her sensitiveness had been reduced to such an extent that she was tolerant of the small amounts of milk and eggs used in the cooking of ordinary foods, so that she has been able since treatment (now two years) to live in comfort on an ordinary diet when she simply avoids the drinking of considerable amounts of milk and the eating of eggs or beef.

Intracutaneous tests with lactalbumin, beef proteins and egg white at the present time give definitely positive reactions.

CASE 5—A man, aged 44 with uninteresting family and past history, began having dyspepsia and attacks of abdominal pain about six years before coming under observation. His dyspepsia had been practically unremitting during this entire period. He was subject to attacks of pain in the epigastrium, which would come on from two to four hours after eating, and last from six to thirty-six hours. At times, the pain was so severe as to completely incapacitate him. He was subject also to dyspepsia, the symptoms consisting of discomfort in the epigastrium, sour eructations, bloating and occasionally nausea, vomiting and diarrhea. He was subject to hives early in his illness. After these had persisted off and on for several months, he discovered that he could keep himself relatively free of hives if he eliminated milk from his diet. Before coming under my observation he had discovered that he felt better and had less abdominal pain and dyspepsia when he avoided both beef and milk.

Physical Examination—The subsequent course of the disease disclosed only three pathologic conditions of interest, namely, stone in the left ureter, gallstones and sensitiveness to milk and to beef serum. Intracutaneous tests with whole milk and beef serum gave rise to characteristic wheals about 1 cm in diameter.

The patient was put on a diet free of milk and beef. While on this diet he seemed very comfortable. While on a beef and milk free diet he was able to eat without trouble such foods as green vegetables, condiments, nuts, etc., which had previously caused discomfort. He was finally given a meal containing a little rare meat as a test. He experienced pain after this meal, which started after about six hours, and lasted about thirty-six hours. He refused to try further experiments of the sort.

The patient was then given a course of inoculations with beef serum and skimmed milk (from 0.001 mg to 1 mg) over a period of six weeks. At the end of this time he was able to take small amounts of beef or milk by mouth.

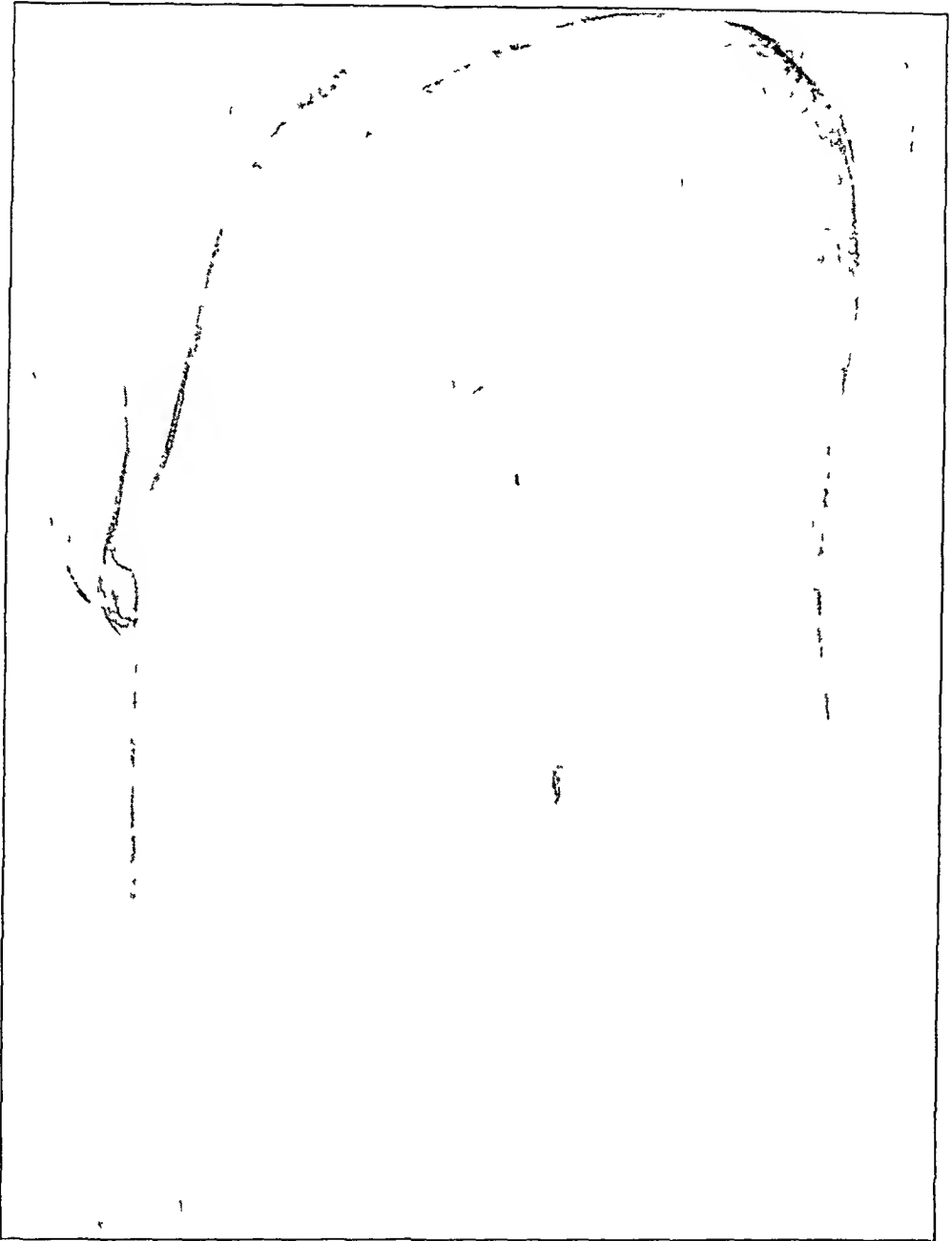


Fig 2—Positive intracutaneous reaction (above) made by the intracutaneous injection of 0.02 mg of lactalbumin. The test appeared in less than four minutes, and gave rise to an irregular wheal, which itched slightly and was replaced by a red edematous area of about the same size which persisted for forty-eight hours. A cutaneous reaction (below) made at the same time by the application of powdered lactalbumin to a scarified area on the skin, with addition of a tenth normal solution of sodium hydroxide solution gave rise to no wheal whatever.

without discomfort and without the appearance of hives. He was then put on a bland diet with gradually increasing quantities of milk, until finally he was able to take $1\frac{1}{2}$ quarts daily without discomfort.

Intracutaneous tests are now (two years later) still positive for lactalbumin and beef albumin.

The subsequent history of the case is of no interest in this connection. The kidney stone passed and the gallstones were removed by operation.

CASE 6—Female, aged 51, referred by my office associate, Dr L. S. Milne, had consulted him on account of severe attacks of abdominal pain, purpura and angioneurotic edema. Her mother was subject to asthma late in life. Her father and a paternal uncle and aunt were subject to attacks of hives and angioneurotic edema, resembling the attacks complained of by the patient.

Previous Illnesses—The patient had been subject to asthma fifteen years ago, she had had stomach and intestinal trouble during her entire life, and she had discovered many years ago that she was unable to eat several foods without becoming violently ill. Those of which she was most certain were rice, beef, milk and eggs. She tested herself under a physician's advice by eating a small amount of rice. Within a few moments she experienced a sensation of extreme weakness, followed in less than one-half hour by abdominal pain, which was agonizing, with nausea, vomiting, diarrhea and mucus stools. A few hours later she had an attack of hives, angioneurotic edema and subcutaneous hemorrhages. She believed similar symptoms had been caused on different occasions by the ingestion of meat and milk, but that they had not appeared so quickly.

The history was otherwise uninteresting, except in that several years previously, in search of relief, the appendix and ovaries had been removed. The symptoms were not noticeably changed by this.

Physical examination disclosed several abnormalities which were of little or no interest in this connection, namely, a slight grade of arthritis, irregular pupils, a slight grade of hypertension with cardiac hypertrophy, a slight trace of albumin in the urine, and a suspicious Wassermann reaction.

Cutaneous tests for sensitiveness were made by applying the powdered proteids of milk, egg, rice, asparagus, cabbage, bean and potato to a scarified area on the skin, but gave practically no reaction. Intracutaneous tests, however, made with casein, lactalbumin, egg white and the proteids of rice, cabbage, beans and potato gave rise immediately to wheals from 1 to 2 cm in diameter, surrounded by large erythematous areas. Within twenty-four hours, the wheals reached the diameter of from 2 to 5 cm and large areas of angioneurotic edema appeared on the left hand and hip. After a second twenty-four hours, the wheals disappeared. A few hours later the patient experienced abdominal pain which required one-half grain of morphin for relief. During the attack there was slight distension of the abdomen, extreme general abdominal tenderness and muscle spasm. The abdominal muscles were held rigidly on account of the pain. The pulse was accelerated, but the temperature and leukocyte counts were normal. Roentgen-ray examination of the stomach during the attack showed the same general features observed at a previous examination when she was free from pain, except for the fact that there was an increase in tone and an increase in the number and depth of the peristaltic waves.

A second attack, similar in every detail and in time, was brought on a week later by the injection of 0.01 mg of lactalbumin.

This case of Henoch's purpura probably does not belong exactly in the same clinical group as those previously reported. It is described for the purpose of showing how violent may be the gastric and cutaneous reactions caused by the ingestion or injection of proteins to which an individual is hypersensitive.

DISCUSSION

I do not wish in this paper to discuss broadly the subject "allergy." I wish, rather, to confine my discussion and report to the concise fact that a reaction caused by hypersensitiveness to a food can give rise to severe abdominal pain, and that pain may be the prominent, and often the sole striking manifestation of the reaction. The abdominal symptoms of the reaction may be very violent, and can easily be mistaken for those caused by a serious abdominal lesion.

I have observed a number of patients who have been sensitive to one or more of the following foods: egg white, egg yolk, shad roe, lactalbumin, casein, beef, pork, honey, strawberries, lettuce, almonds, beans, onions, cabbage, rice, potatoes, tomatoes, paprika and pimento, and who have invariably had an attack of severe abdominal pain whenever they have eaten the foods to which they were sensitive. In the majority of the cases pain appeared soon after ingestion of the food and lasted from three to six hours. In several cases, however, it did not appear for from three to twenty-four hours, and in these cases, it lasted much longer. In a majority of the cases pain was associated with nausea and vomiting, less frequently with indigestion, bloating, diarrhea, and mucus stools, and much less frequently with hives, angio-neurotic edema and purpura.

The pain complained of was evidently the result of a reaction caused by contact between the gastro-intestinal mucosa and the food product to which it was sensitive. This contact gave rise to gastro-intestinal symptoms in much the same way that contact of the mucous membrane of the respiratory tract with a pollen to which it is sensitive gives rise to symptoms of hay-fever or asthma. In other words, the pain and other gastro-intestinal symptoms described seem fundamentally analogous in pathogenesis to the symptoms hay-fever and asthma. An attack of asthma can be brought on in a sensitive individual by a subcutaneous injection of the pollen to which he is sensitive. Analogous to this, several of the patients had gastro-intestinal pain after subcutaneous injections of an extract of the food to which they were sensitive.

An experience of interest in this connection might be mentioned here showing that allergy, caused even by the injection of pollen extract in the treatment of hay-fever and asthma, can cause abdominal pain as part of a general reaction. A minority of patients who suffer from asthma and hay-fever complain of dyspeptic symptoms which appear at the beginning of the hay-fever season each year and disappear with the first frost. Occasionally, such a patient experiences abdominal pain in addition to the ordinary dyspeptic symptoms. One such patient, while under treatment with pollen extract, experienced abdominal pain

after each inoculation of pollen, which was so severe that he refused treatment after the third inoculation on this account. The pain was severe, steady, nonradiating and localized in the epigastrium. It appeared a few minutes after each inoculation and lasted several hours. It was not accompanied by other symptoms of allergy, in fact, in this case it was the sole manifestation of reaction to the pollen.

It is interesting to mention that among the patients having food allergy observed by me, nearly 50 per cent had demonstrable pathologic lesions in the alimentary tract or in its appendages. These lesions were recurrent appendicitis, gallstones, duodenal ulcer, dense adhesions and extreme ptosis. Food allergy, like other forms of allergy, is, perhaps, primarily dependent on an inherited constitution which renders the individual susceptible of becoming hypersensitive to certain alien substances. It seems quite possible, however, that an abnormality in the alimentary tract may be a contributory factor in the etiology of this type of case.

It is furthermore of interest to note the fact that in two cases attacks of acute appendicitis which required surgical intervention, followed attacks of food allergy. While it is hardly justifiable to draw conclusions from two cases it is justifiable to say that alimentary allergy is a very real cause of gastro-intestinal turmoil. It may cause violent muscular contractions, pathologic secretions, edema of the mucous membrane and local anemia. It is very reasonable to suppose that this may occasionally set up infection and inflammation and give rise to serious disease in the alimentary tract or in its appendages.

DIAGNOSIS

In the diagnosis of alimentary allergy as the cause of abdominal pain the history of the case is all important. If a patient invariably has pain and other gastro-intestinal symptoms after eating certain articles of food, and is at other times free of digestive disturbances, the diagnosis is simple and easy. If, with this there should be other manifestations of allergy, such as hives, angioneurotic edema, or asthma, the diagnosis is comparatively certain. It can further be verified in the great majority of cases by the use of intracutaneous tests with the suspected proteids. Skin tests in this particular type of allergy are not always so clearly positive as they are in hay-fever and asthma, but in almost every case characteristic wheals can be obtained if intracutaneous tests are made with the correct proteins.

In making a diagnosis of food allergy it is not advisable to accept a patient's statement for the fact that the eating of a certain food invariably causes abdominal pain. Patients are often mistaken about this. It will often be found on putting them to a test and having them eat the suspected food that it causes no disturbance whatever.

When an individual is hypersensitive to an uncommon article of diet, such as shad roe or honey, the diagnosis of the condition is

easy and is often made by the patient himself. He usually gives a history of occasional attacks of pain and vomiting, which last a few hours, and usually states that he is free of digestive disturbance at other times. When he is sensitive to a common food, such as eggs or milk, however, the diagnosis may be very difficult. Milk and eggs are used so commonly in cooking, that a patient who is highly sensitive to either one may be in a reactive state almost constantly, and may have such symptoms as pain after eating, nausea, vomiting, bloating and indigestion almost every day. He may be unable to place the blame on any one particular food. The diagnosis may be doubly difficult because of the fact that the gastro-intestinal mucous membrane, when in a chronically reactive state, is often very irritable, with the result that rough, or stimulating, foods, such as coarse vegetables, fruits, nuts, condiments, alcohol, etc. irritate the stomach and augment the symptoms. This usually leads a patient to place the blame for his trouble on foods of this variety rather than on the primary offenders, milk or eggs. It is in cases of this type that cutaneous tests have their greatest sphere of usefulness. One can occasionally discover the offending proteid by having the patient eliminate first one and then another common article of diet for a period of a week or more. By keeping careful notes, he may be able to discover on which diet he is most comfortable and then by eating a suspected food determine whether or not it actually causes distress. This method of diagnosis may be very slow, however, and may even fail utterly should the patient be sensitive to two or more common articles of food.

CUTANEOUS TESTS

Cutaneous tests are not essential to the diagnosis of food allergy when an individual is sensitive to one unusual article of diet, such as honey or shad roe, and has pain soon after the eating of certain foods. In cases of this sort, the diagnosis can usually be made without a cutaneous reaction. The great sphere of usefulness for cutaneous tests is in patients who are sensitive to a common article of diet, such as milk or eggs, or who are sensitive to several articles of diet, or for patients who have delayed reactions and experience no discomfort for a number of hours after the ingestion of a food. For the correct and prompt diagnosis of cases of this type cutaneous tests are indispensable.

Several methods of making cutaneous tests are now being used. A method described by Walker is the simplest and most convenient. It consists of applying the suspected proteids in dry powdered form to a scarified area on the skin and placing on this a small drop of tenth normal sodium hydroxide solution. The appearance of a wheal indicates a positive reaction.

This method is very satisfactory in the majority of hay-fever and asthma cases. It failed almost completely, however, in a number of cases of food allergy observed by me which gave very large wheals after intracutaneous injections of a solution of the suspected proteids. Intracutaneous tests are not so handy as the scarification method because of the fact that test solutions tend to lose their potency and it is quite difficult and laborious to keep a large number of potent solutions on hand. I have used a very simple means of making intracutaneous tests. It can be carried out accurately without much trouble or expense.

INTRACUTANEOUS METHOD

Place about 0.1 mg. of each of the suspected proteids (in dry powdered form) to be used in the tests on a large sterile plate. One-tenth mg. of a powdered protein can be guessed at crudely as the amount of powder which can be taken up easily on the end centimeter of a Peerless round wood applicator. To each proteid add 0.1 cc. of physiologic sodium chlorid solution (distilled water will not dissolve globulin). Stir each carefully with separate wooden applicators until the proteids go into solution. Draw up the first solution to be used in a tuberculin syringe armed with a small needle, and inject intracutaneously 0.02 cc. (0.02 mg. of proteid). Wash the syringe carefully in each of three separate vessels containing sterile physiologic sodium chlorid solution, numbered 1, 2 and 3. If care is used, the last washing should remove every trace of proteid used in the previous test. The same syringe may then be used for the second test and the same wash waters can be used several times, that is, if the vessels are always used in rotation according to their numbers—1, 2 and 3.

This method has several advantages. First, it is likely to give a larger number of definite positive reactions than scarification tests. Second, dry proteins keep indefinitely. Third, it is simple.

The scarification and intracutaneous methods are compared in Figures 1 and 2. Figure 1 represents a patient (Case 1) on whom the two methods gave exactly the same result. Figure 2 represents a patient (Case 6) on whom a cutaneous test was practically negative, whereas an intracutaneous test gave rise immediately to a definite wheal.

In a normal person nothing striking follows an intracutaneous injection of a foreign protein. If an individual is inoculated with a proteid to which he is sensitive, however, in a few moments a wheal will usually appear which varies in size from 0.5 to 2 cm. or more in diameter. The wheal is usually paler than the skin, usually shows pseudopod-like projections, is often surrounded by an irregular erythematous area, and often itches intensely.

The wheal usually appears and reaches its minimum size within a few minutes. It may not reach its maximum size for several hours, however. If it appears quickly, it usually disappears within one or two hours. If it reaches its maximum more slowly, it usually lasts much longer.

Cutaneous tests are not so striking in the average case of food allergy as they are in patients who have hay-fever and asthma. In the pollen cases, the scarification method is almost always adequate, and, as a rule, gives rise to a large wheal within a few minutes if the correct pollen is used. In food cases, however, an intracutaneous test is very often necessary for a convincing reaction.

TREATMENT

The treatment of food allergy may be considered from three points of view. First, removal of the cause, second, protein treatment, third, symptomatic treatment.

Removal of the cause is very simple when a person is sensitive to a rare article of food, such as honey, shad roe, strawberries, beans or paprika. Nothing is easier than to instruct a patient to cease eating such articles and to live in comfort. When an individual is sensitive to a food used so commonly in cooking as eggs or milk, however, removal of the cause is by no means easy. Unfortunately, a person is often so highly sensitive that a mere trace of the food to which he is sensitive suffices to make him miserable. He may find it necessary to be constantly on the lookout to avoid taking such a trace unknowingly. For this reason, protein therapy in a case of this sort is advisable. It must be mentioned here that in treating a case of this type, it is not necessary to obtain complete desensitization in order to relieve the patient. Complete desensitization may not be possible, and an effort to attain this result may not be advisable or really very desirable. If a patient's sensitiveness is reduced to a point where he can simply tolerate the small amounts of the food which are used in cooking other foods, he can, with a little care, live a life of comfort. For example, if a patient were sensitive to casein, and his sensitiveness were reduced to such a point that he could tolerate the amount of casein encountered in ordinary cooked foods, such as bread, cake, potatoes, etc., he could, by avoiding the drinking of milk and the eating of foods consisting chiefly of milk, live in comfort. It is not possible here to discuss broadly the subject of protein therapy. For this the many splendid articles already published should be consulted.

As symptomatic or temporary measures might be mentioned a bland diet, such as that used for hyperacidity, avoiding, of course, the proteins to which the patient is sensitive, sodium bicarbonate, in doses sufficient to reduce hyperacidity, atropin, and in the severer cases, removal of the exciting agent by gastric lavage and purgation and epinephrin or morphin subcutaneously, if needed.

SUMMARY AND CONCLUSION

It is well known that humans and animals may become sensitive to alien bodies of many varieties. When this is the case, they react when-

ever they come in contact with the body to which they are sensitive. The gastro-intestinal mucosa may become hypersensitive to an article of food with the result that the patient experiences severe abdominal pain, often associated with nausea and vomiting, whenever he eats the food to which he is sensitive. These alimentary symptoms are in many cases the sole striking manifestations of the reaction. Individuals sensitive to uncommon articles of diet, such as shad roe, lettuce, honey, strawberries, cabbage, tomatoes, paprica, etc., usually give a history of occasional attacks of abdominal pain and digestive upset. They are, as a rule, free of digestive disturbance between attacks. Individuals sensitive to the commoner articles of food, such as milk or eggs, have more frequent attacks of pain and are often subject to chronic indigestion as well.

The symptoms of food allergy may simulate somewhat those of a surgical lesion in the abdomen, and an error in diagnoses can be made unless this condition is kept in mind.

Food allergy was observed more frequently in individuals who had organic lesions in the gastro-intestinal tract or in its appendages than it was in normal persons.

Food allergy had acute appendicitis as a sequel in two cases, and surgical intervention was required.

BIBLIOGRAPHY

- Arthus, M. Injections Repetees de serum de cheval chez le lapin, *Compt rend Soc de biol Par* **55** 817, 1903. Anaphylaxie et immunité, *Compt rend Acad d sc, Par* **154** 1363, 1912. Anaphylaxie passive du lapin, *Compt rend Soc de Biol* **82** 412, 1919.
- Besredka, A, and Gloyne, S. R. Anaphylaxis and Anti-Anaphylaxis and Their Experimental Foundation, St. Louis, C. V. Mosby Company, 1919.
- Blackfan, K. D. A Consideration of Certain Protein Hypersensitiveness in Children, *Am J M Sc* **160** 341, 1920.
- Boughton, T. Anaphylaxis and Allied Phenomena in Relation to Disease, *J Lab & Clin M* **5** 597, 1920.
- Boughton, T. H. Studies in Protein Intoxication, *J Immunol* **1** 105, 1916, **2** 501, 1917, **4** 213, 1919.
- Coca, A. F., and Kosakai, M. Studies in Anaphylaxis, *J Immunol* **5** 297, 1920.
- Coca, A. F. Mechanism of Anaphylaxis Reaction in Rabbits, *J Immunol* **4** 219, 1918. Hypersensitiveness. Anaphylaxis and Allergy, *J Immunol* **5** 363, 1920.
- Cooke, R. Protein Sensitization in the Human, with Special Reference to Bronchial Asthma and Hay-Fever, *M Clin N America* **1** 721, 1917.
- Engman, M. F., and Wander, W. G. Application for Cutaneous Sensitization to Diseases of the Skin, *Arch Dermat & Syph* **3** 223, 1921.
- Gay, F. P. and Southard, E. E. On Serum Anaphylaxis in the Guinea-Pig, *J M Research* **16** 143, 1907, **18** 407, 1908, **19** 1, 5, 17, 1918.
- Joltrain. Treatment of Alimentary Anaphylaxis, *Bull et mem Soc med d hop de Par* **43** 556, 1919.
- Lowdermilk, R. C. Hay-Fever, *J A M A* **63** 141 (July 11) 1914.
- Laroche, G. and Richet, C. L'anaphylaxie alimentaire etat actuel de la question, *Bull med Par* **34** 625, 1920.

- McBride, W L, and Schorer, E H Erythematous and Urticarial Eruptions Resulting from Sensitization to Certain Foods, *J Cutan Dis* **34** 70, 1916
- Novy, F G, and DeKruif, P H Anaphylatoxin and Anaphylaxis, *J A M A* **68** 1524 (May 26) 1917
- Otto, R Das Thcobald Smithsche Phaenomen der Serum-Ueberempfindlichkeit, *Gdnkschr f d Verstorb Generalstabsarzt d Armee* **1** 153, 1906
- Von Pirquet, C, and Schick, B Theorie der Inkubationszeit, *Wien klin Wchnschr* **16** 753, 1903
- Pagniez, P, and Vallery, R Digestive Anti-Anaphylaxis, *Ann med, Par* **8** 303, 1920
- Rosenau, M J, and Anderson, J F A Study of the Cause of Sudden Death Following the Injection of Horse Serum, *U S Hygienic Lab Bull*, No 29, 1906 Studies on Hypersusceptibility and Immunity, *ibid*, No 36, 1907 Further Studies on Anaphylaxis, *ibid*, No 45, 1908
- Rosenau, M J Studies in Food Poisoning An Experimental Lunch with Canned Food Containing Bacteria, *M Clin N America* **3** 913, 1920
- Rosenau, M J, and Anderson, J F Hygienic Lab Bull, No 29, 1906, No 36, 1907, No 45, 1908
- Vaughan, V C Protein Split Products in Relation to Immunity and Disease, Philadelphia, Lea & Febiger, 1913
- Vaughan, V C, and Wheeler, S M The Effects of Egg-White and Its Split Products on Animals a Study of Susceptibility and Immunity, *J Infect Dis* **4** 476, 1907
- Walker, I C Studies on the Cause and Treatment of Bronchial Asthma, *J A M A* **69** 363 (Aug 4) 1917 Causation of Eczema, Urticaria and Angio-neurotic Edema by Proteins Other Than Those Derived from Foods Study XVIII, *J A M A* **70** 897 (March 30) 1918 Bronchial Asthma, *Arch Int Med* **22** 466 (Oct) 1918 Studies on the Sensitization of Patients with Bronchial Asthma, Studies I-V, *J M Research* **35** 373, 391, 487, 497, 309, 1917, Studies VI-VIII, *J Immunol* **2** 227, 237, 243, 1917, Study IX, *Am J Bot* (July) 1917, Studies X-XIV, *J M Research* **36** 231, 237, 245, 423, 1917, Studies XV-XVII, *ibid* **37** 57, 377, 287, 1917
- Wells, H The Present Status of the Problems of Anaphylaxis, *Physiol Rev* **1** 44, 1921
- White, C J Two Modern Methods to Be Employed in the Treatment of Chronic Eczema, *J A M A* **68** 81, 1917
- Zinsser, H The More Recent Developments in the Study of Anaphylactic Phenomena, *Arch Int Med* **16** 223 (Aug) 1915

PHENOLS IN THE URINE IN PELLAGRA ~

M X SULLIVAN AND PAUL R DAWSON

SPARTANBURG, S C

Indican, taken as a measure of putrefaction in the intestines, has been reported repeatedly in the urine of persons suffering from pellagra¹

Myers and Fine, working in cooperation with the Thompson-McFadden Commission, concluded that indicanuria is excessive in the cases with gastric inefficiency Hunter, Givens and Lewis, however, showed that gastric inefficiency is only one of the factors involved in indican formation A very important factor is to be found in the patient's diet—the tendency to excessive indican formation being greater on a meat diet They showed further that the existence of pellagra is compatible with any degree whatever of indican production, even none at all, and that indicanuria, undeniably a common feature of the disease, is by no means, an essential one

That indicanuria is not an essential factor in pellagra is also shown by the findings of Goldberger and Wheeler Thus, in 1915, Goldberger and Wheeler² showed that pellagra could be produced experimentally in previously healthy human subjects by feeding a restricted one-sided, mainly cereal diet of the type found to be associated with a high incidence of pellagra The urine of their subjects³ on the pellagra producing diet, though in some cases, occasionally showing indican, was, on the whole, predominantly free from indican

Though indican has been reported in pellagra, especially on a meat diet, little attention has been paid to the products of intestinal putrefaction, such as the phenols As to the phenols, Folin and Denis⁴ say As an index or measure of such putrefaction, the phenol products determined by our scheme of analysis may fairly be considered at least as important as the indican determinations, which heretofore, have been used almost exclusively as measures of intestinal putrefaction

* From the U S Public Health Service

1 Marie Pellagra, translated by Lavinder and Babcock, Columbia, S C p 186, Translator's Note Tucker J A M A **56** 246, 1911 Lavinder A Precis Public Health Bull, 48, 1912 Myers and Fine Am J M Sc **145** 705, 1913 Hunter, Givens and Lewis Bull 102, II, Hygienic Lab Washington, D C, 1916 Wilson and Roaf Report of a Committee of Inquiry Regarding the Prevalence of Pellagra Among Turkish Prisoners of War, Cairo, Egypt 1918

2 Public Health Reports **30** No 46, Nov 12, 1915

3 Bull 120, Hygienic Lab, Washington 1920

4 J Biol Chem **22** 309, 1915

In 1920, determinations were made on the urine of the patients at the Pellagra Hospital for phenols, total, free and conjugated. The patients with a few exceptions were rather mild though distinct cases of pellagra. Three types of diets were employed (1) Standard, which was a broad nourishing diet, (2) Rankin Farm experimental diet, plus milk, which contained the ingredients employed by Goldberger and Wheeler in producing experimental pellagra in man—with the addition of sweet milk, (3) Rankin Farm experimental diet plus reconstructed skimmed milk made from skimmed milk powder. These diets were employed at the hospital by Wheeler, in the study of the value of sweet milk and powdered skimmed milk in the treatment of pellagra. These diets, qualitatively, are as follows:

STANDARD	RANKIN FARM
Cereals (rice, oatmeal)	Biscuits
Bread (corn, wheat, soda biscuits)	Fried mush
Milk (sweet and buttermilk)	Grits
Meats (chicken, beef, pork, lamb)	Brown gravy
Fish	Corn bread
Eggs	Sweet potatoes
Bananas, prunes, apples, or oranges	Cabbage
Tomatoes, Irish and sweet potatoes, greens,	Turnip tops
peas and beans	Rice
Coffee and sugar	Sugar
	Syrup
	Coffee
	Sweet milk in diet (2)
	Reconstructed skimmed milk in diet (3)

The determination of the phenols in the twenty-four hour urine was made according to the colorimetric method of Folin and Denis.⁵ Of the twenty-four hour urine, 10 c c were placed in a 50 c c volumetric flask. To this urine were added about 10 c c of a 5 per cent silver lactate solution in 5 per cent lactic acid. A few drops of colloidal iron were then added and the mixture shaken well and then made up to a volume, and filtered. The filtrate, thus freed from uric acid and traces of protein, was freed of silver by precipitation with a saturated solution of sodium chlorid containing 10 c c of concentrated hydrochloric acid per liter, and filtering. Of the clear filtrate, suitable aliquot samples were measured out with a pipette for determination of the free and total phenols. The amount used would vary with the degree of dilution of the material, but in most cases 25 c c were satisfactory. For determination of free phenols this amount was transferred to a large test-tube, 15 drops of concentrated hydrochloric acid added, a few beads, and the mouth of the tube closed with a small funnel. The contents of the tube were then brought

⁵ J Biol Chem **12** 239, 1912, **22** 306, 1915

to boiling over a free flame and heated for ten minutes in a boiling water bath. After cooling, the preparation was transferred to a 100 c c volumetric flask.

To another 100 c c volumetric flask was added accurately 10 or 20 c c of the phenol standard. This standard consisted of a solution of 0.581 gm resorcinol in 1 liter fifth normal hydrochloric acid, as recommended by Benedict and Theis,⁶ and is stated by them to be equivalent in color production to a pure phenol standard of 0.500 gm per liter.⁷ To the free phenol preparation in the 50 c c flask was added 5 c c of the phenol reagent of Folin and Denis, and 15 c c of the saturated solution of sodium carbonate, and to the total phenol preparation and standard in the 100 c c flask was added 10 c c of the phenol reagent and 25 c c of the saturated solution of sodium carbonate. The preparations, after thorough mixing, warming up slightly on a water bath, standing about twenty minutes, and making up to volume, were filtered and compared in the Duboscq colorimeter. The results were calculated in the usual manner, taking into consideration the strength of the standard, the difference between the volumes of the free phenol preparation and the standard, and the proportion of the original volume of material represented by the sample. The results for the estimation of phenols in the urine of pellagrins, of seven non-pellagrous subjects under observation in the hospital, and of two normal nonpellagrous workers in the laboratory are given in Tables 1, 2, and 3, respectively. No tests were made on the phenol content of the feces for, as shown by Folin and Denis,⁸ the phenol content of the feces is small in comparison to the phenol content of the urine.

The data given in Tables 1, 2, and 3, show that, as a rule, the phenol excretion (total, free, and conjugated) by the pellagra patients studied does not surpass that of the controls, though three subject cases (45, 47, and 49) did show an increase in urinary phenol. Between the active pellagra stage and the convalescent stage, likewise, there is, as a rule, very little difference. Two subjects (Cases 51 and 58) give a greater excretion of total phenol in the convalescent stage than in the active period, but this difference is explained by the fact that in the active pellagra period the patient suffered from diarrhea. The percentage of conjugation is, as a rule, practically the same for the patients in the pellagra stage and in the convalescent stage when they were about to be discharged and for the normal people.

Of the sixteen patients listed in Table 1, eight were males, eight were females. The average excretion of phenols in the males in the active

6 Benedict and Theis. *J Biol Chem* **36** 95, 1918.

7 By direct color comparison with a standardized solution of pure phenol, the resorcinol standard made by us was found to be equivalent to 0.5788 gm phenol per liter. The cause of this discrepancy has not been determined.

8 *J Biol Chem* **26** 507, 1916.

TABLE 1—PHENOLS AND INDICAN IN THE URINE IN PELLAGRA (a) IN PELLAGROUS STAGE, (b) IN CONVALESCENT STAGE

Case No	Sex	Date of Admission	Date of Discharge	Duration of Tests	Status of Pellagra	Volume of Urine, Cc	Total Nitrogen of Urine Gm	Total Phenol, Gm	Free Phenol, Gm	Conjugated Phenol, Gm	Per Cent Conjugation	Indican	Diet
45	M	4/3	4/7	4 1/4	Mild	1 080	a 8.934	a 0.613	a 0.289	a 0.325	a 52.9	1	Standard
47	F	4/5	5/8	4/6-5/8	Mild	a 1,050	a 12.097	a 0.654	b 0.362	a 0.292	a 44.7	a	Standard
49	M	4/23	5/29	4/24-5/28	Moderate	b 1,727	b 15.578	b 0.547	a 0.265	b 0.283	b 51.6	b	Standard
51	M	4/29	7/17	5/1-7/17	Severe, complicated	a 1,120	a 11.46	a 0.733	a 0.416	a 0.337	a 44.8	a	Standard
53	M	5/8	6/1	5/10-5/28	Mild	b 1,553	b 12.346	b 0.468	b 0.198	b 0.269	b 57.6	b	What patient would eat
55	M	5/11	6/11	5/12-6/11	Mild	b 1,520	b 9.453	b 0.460	a 0.198	a 0.102	a 34.0	1	Standard
56	F	5/12	6/18	5/13-6/18	Mild	a 1,310	a 4.881	a 0.196	b 0.258	b 0.202	b 43.9	1	What patient would eat
59	M	5/17	7/3	5/18-7/2	Mild	a 1,780	a 9.559	a 0.400	b 0.287	a 0.065	a 33.5	b	Standard
62	M	5/21	6/11	5/23-6/11	Moderate	b 1,320	b 9.682	a 0.347	a 0.174	a 0.173	a 49.8	1	Standard
63	M	5/21	6/12	5/23-6/11	Mild	a 800	a 8.212	b 0.296	b 0.119	b 0.117	b 59.8	b	Rankin farm + milk
67	F	6/6	7/31	6/7-7/30	Severe	b 790	b 5.713	a 0.235	a 0.121	a 0.114	a 48.5	1	Standard
68	T	6/7	7/31	6/7-7/30	Severe	a 1,350	a 8.288	b 0.143	b 0.084	b 0.058	b 40.8	b	Rankin farm + milk
65	F	6/1	7/17	6/4	Mild, mental symptom	b 1,014	b 7.154	a 0.386	a 0.205	a 0.182	a 47.0	a	Standard
70	T	6/8	6/26	6/8-6/26	Severe dermal type	a 810	a 11.042	a 0.313	a 0.205	a 0.198	a 34.5	1	Rankin farm + pow d milk
74	F	6/11	7/24	6/18-7/23	Severe systemic type	b 1,115	b 7.373	b 0.397	b 0.226	b 0.171	b 42.9	b	Rankin farm + milk
76	T	6/17	7/24	6/21-7/23	Severe systemic	a 1,650	a 10.963	a 0.429	a 0.220	a 0.209	a 48.7	1	Standard
Average of a													
Average of b													

TABLE 2—PHENOLS IN URINE OF NON-PELLAGROUS PATIENTS (a) AT ADMISSION, (b) AT DISCHARGE

Case No	Sex	Date of Admission	Date of Discharge	Duration of Tests	Clinical Condition	Volume of Urine, Cc	Total Nitrogen of Urine, Gm	Total Phenols, Gm	Free Phenols, Gm	Conjugated Phenols, Gm	Per Cent Conjugation	Diet
48	F	4/17	4/24	4/18	Ichthyosis Malnutrition, gonorrhea, diarrhea Malnutrition constipation Eczema	a 1,675	a 8.201	a 0.288	a 0.167	a 0.121	a 42.0	Standard
50	F	4/23	5/12	4/24		a 870	a 2.666	a 0.233	a 0.100	a 0.073	a 31.3	Standard
54	F	5/ 9	5/14	5/10		a 1,520	a 6.079	a 0.196	a 0.126	a 0.070	a 35.7	Standard
78	F	5/16	6/12	5/18-6/11	Cystitis, hernia Mental case, slight desquam on hands Diarrhea, headaches	a 600	a 4.234	a 0.134	a 0.077	a 0.037	a 42.5	Standard
64	M	5/31	6/11	6/ 2-6/11		b 1,260	b 8.455	b 0.308	b 0.128	b 0.180	b 58.4	Standard
66	F	6/ 1	6/19	6/ 2-6/18		a 1,720	a 10.581	a 0.290	a 0.162	a 0.128	a 44.1	Standard
73	F	6/ 9	7/10	6/10		b 1,440	b 9.618	b 0.292	b 0.145	b 0.137	b 50.3	Standard
						a 920	a 7.193	a 0.216	a 0.113	a 0.103	a 47.7	Standard
						b 1,640	b 9.433	b 0.362	b 0.200	b 0.162	b 44.8	Rankin farm and milk
						a 1,080	a 5.561	a 0.287	a 0.172	a 0.115	a 40.1	Rankin farm and milk
Average		a 1,198	a 6.356	a 0.235	a 0.140	a 0.095	a 40.5					
		b 1,450	b 9.132	b 0.321	b 0.158	b 0.163	b 51.2					

stage is 0.417 gm, by the females, 0.276 gm. The average total nitrogen of the twenty-four hour urine is 9.044 gm for the males, and 6.337 gm for the females. It would seem, then, that as shown by Folin and Denis the phenol excretion tends to be higher, the higher the protein intake. Similarly, there is a tendency, on the whole, for a greater excretion of phenols on the more generous Standard diet than on the Rankin Farm diet plus milk. However, when the total nitrogen and phenols of the active pellagra stage are compared, with the total nitrogen and phenols of the convalescent stage, it may be seen from Table 1 (a and b average figures) that, on the whole, there is but little increase of total phenols with the increase in total nitrogen.

The nonpellagrous group in Table 2, with malnutrition and skin diseases not pellagrous, had a lower excretion of phenols than the true pellagra patients and than the normal people. This lower excretion of total phenols by Group 2 may be explained by the facts that, with

TABLE 3—PHENOLS IN URINE OF NORMAL INDIVIDUALS

Subject	Volume of Urine, Cc	Total Nitrogen of Urine, Gm	Total Phenols, Gm	Free Phenols, Gm	Conjugated Phenols, Gm	Per Cent Conjugation
1 Active healthy adult	740	9.771	0.293	0.134	0.158	54.1
	880	11.917	0.303	0.175	0.128	42.1
2 Active healthy adult	1,370	11.174	0.340	0.186	0.154	45.2
	1,420	11.431	0.276	0.153	0.118	42.6
	1,800	12.386	0.249	0.145	0.104	41.6
	1,550	12.357	0.313	0.184	0.129	41.1
	1,300	15.213	0.470	0.226	0.244	51.9
	890	12.900	0.297	0.133	0.114	38.4
Average	1,244	12.144	0.318	0.174	0.144	44.6

the exception of Subject 64, a man, 75 years of age, these subjects were females, and were also on a lower level of protein metabolism and, what was probably of greater influence on the urinary phenol, had more or less looseness of the bowels.

Folin and Denis have shown that from 0.3 to 0.5 gm total phenols in a twenty-four hour quantity of human urine is not abnormal. On the basis of the conclusion, we should judge that in three out of sixteen cases, or approximately 19 per cent, there was an increase in total phenols in the active stage of pellagra and that in the other cases the phenol excretion was well within normal limits.

Of the fourteen cases in which the urinary phenols were determined from entrance to discharge from the hospital, seven (Cases 51, 53, 62, 68, 70, 74, and 76) show a varying degree of increase in the total phenols of the urine on discharge as compared with that on entrance, six (Cases 47, 49, 55, 56, 59, and 63) have a varying degree of decrease at discharge as compared with entrance, while one subject (Case 67) has the same quantity of total phenols in the urine at discharge as at

entrance. The conclusion may be drawn, then, that there is little correlation between the extent of phenol excretion in the urine and the pellagious condition.

Of the sixteen cases listed in Table 1, the urine of twelve was tested for indican by Miss Catherine Maiden of this station. Ten gave a very marked test for indican in the active pellagra period and are listed as having an increased formation of indican. It would seem that while the phenol excretion in the urine is in the main normal, there is a tendency for increased formation of indican. The generally normal phenol excretion with the tendency to increased indican excretion would imply that whatever putrefaction occurs in the intestines of pellagrins takes place rather high up in the intestines, as suggested by Myers and Fine.

CONCLUSIONS

The general conclusion as to the phenol excretion of the pellagra patients studied by us, mild cases for the most part, is that there is little variation from the normal either in amount of total phenols or in percentage of conjugation. Only three out of sixteen patients showed an increase in phenols in the active stage of the disease. There is some evidence that the phenols are increased with increased protein metabolism.

Of twelve cases tested for indican, ten gave a marked test. What putrefaction occurs in pellagra on the diets employed seems to take place rather high up in the intestines.

THE BASAL METABOLISM AND THE SPECIFIC DYNAMIC ACTION OF PROTEIN IN LIVER DISEASE *

JOSEPH C AUB, M D AND JAMES H MEANS, M D
BOSTON

Much study of the liver has shown that it has so large a factor of safety that even when quite severely injured by disease it is still capable of performing its metabolic functions adequately. These studies have nearly all been made from the point of view of excretion, however, and but little work has been done on the speed of absorption and the utilization of foods in liver disease. With the disturbances of circulation found in cirrhosis, and the marked loss of liver tissue, it seems reasonable that, at least, the speed of utilization of foodstuffs might be delayed. This can best be studied by the respiratory metabolism, and in this paper, therefore, the question of total metabolism and of utilization of protein in liver disease is discussed.

The specific dynamic action of protein is a term first given by Rubner¹ to the increase in total heat production which follows the ingestion of large quantities of protein. Various explanations of this fact have been offered, but the work of Lusk² has shown that amino-acids stimulate the body cells to increased heat formation. By feeding glycocoll to a phlorhizinized dog, he showed that the breakdown and metabolism of the acids themselves is not the cause. The total caloric content of the glycocoll was recovered in the urine as glucose and urea, but there was still a distinct specific dynamic action elicited, which must have been a response of the cells of the organism. This work has been confirmed by Grafe³. That this stimulation was not dependent on the mass of striated muscle nor on the surface area was suggested by Aub and DuBois,⁴ who studied the effect of large quantities of meat on a legless man and an achondroplastic dwarf. These subjects with normal torsos, but reduced muscle area, responded with a

* From the Medical Service of the Massachusetts General Hospital and the Medical Department of the Harvard Medical School, aided, in part, by a grant from the Proctor Fund, Harvard University.

1 Rubner, M. *Die Gesetze des Energieverbrauchs bei der Ernährung*

2 Lusk, G. *Animal Calorimetry, An Investigation into the Causes of the Specific Dynamic Action of the Foodstuffs*, J Biol Chem **20** 555, 1915

3 Grafe, E. *Beiträge zur Kenntnis der Ursachen der spezifisch dynamischen Wirkung der Eiweisskörper*, Deutsch Arch f klin Med **118** 1 1915

4 Aub, J C, and DuBois, E F. *The Basal Metabolism of Dwarfs and Legless men, with Observations on the Specific Dynamic Action of Protein*, Arch Int Med **19** 840 (June) 1917

relatively greater increase of metabolism than did the normal controls. This suggested that the effect might be located in the viscera.

DuBois⁵ also showed that the specific dynamic action of protein was normal in exophthalmic goiter and in cretinism, the rise of metabolism being normally superimposed on the abnormal basal rate. Work reported in this article confirms this observation for hyperthyroidism. These results suggest that the thyroid gland is not the seat of the dynamic action.

A third possible organ for the origin of this specific dynamic action is, of course, the liver. There is some evidence brought forward by Salaskin⁶ and Jansen,⁷ and better work by Loeffler⁸ and Van Slyke⁹ that the liver is able to transform amino-acids into urea. But Loeffler¹⁰ has also shown that fasting livers, perfused with a nitrogen-free solution, may form urea. This makes conclusions from this type of work questionable, although Loeffler still believes that urea is formed in the liver from some amino-acids. Fiske and Sumner,¹¹ however, have shown that urea may be formed from amino-acids in an organism with the liver eliminated, which suggests that urea formation from amino-acids is not confined to the liver.

There has been much study of the metabolism in liver disease, from the point of view of amino-acid assimilation and excretion. Bergell and Blumenthal¹² found no reduced catabolism of amino-acids other than leucin and tyrosin in a case of acute yellow atrophy. Feigl and Luce¹³ carefully studied a case of acute yellow atrophy and observed a marked negative nitrogen balance as well as an increased excretion of amino-acids in the urine. The interesting case of yellow atrophy studied by Stadie and Van Slyke¹⁴ showed a marked increase of amino-

5 DuBois, E. F. Metabolism in Exophthalmic Goiter, *Arch Int Med* **17** 915 (June) 1916.

6 Salaskin, S. Ueber die Bildung von Harnstoff in der Leber der Säugethiere aus Amidosäuren der Fettreihe, *Ztsch f physiol Chem* **25** 128, 1898.

7 Jansen, B. C. P. The Function of the Liver in Urea Formation From Amino-Acids, *J Biol Chem* **21** 557, 1915.

8 Loeffler, W. Ueber Harnstoffbildung in der isolierten Warmbluterleber, *Biochem Ztschr* **76** 55, 1916.

9 Van Slyke, D. D. Significance of the Amino-Acids in Physiology and Pathology, *Arch Int Med* **19** 56 (Jan) 1917.

10 Loeffler, W. Desaminierung und Harnstoffbildung in Tierkörper, *Biochem Ztschr* **85** 230, 1918.

11 Fiske, C. H., and Sumner, J. B. The Importance of the Liver in Urea Formation from Amino-Acids, *J Biol Chem* **18** 285, 1914.

12 Bergell, P. and Blumenthal, F. Ueber akute gelbe Leberatrophie, *Charite Ann* **30** 19, 1906.

13 Feigl, J., and Luce, H. Neue Untersuchungen über akute gelbe Leberatrophie, *Biochem Ztschr* **79** 162, 207, 1917.

14 Stadie, W. C. and Van Slyke, D. D. The Effect of Acute Yellow Atrophy on Metabolism and on the Composition of the Liver, *Arch Int Med* **25** 693 (May) 1920.

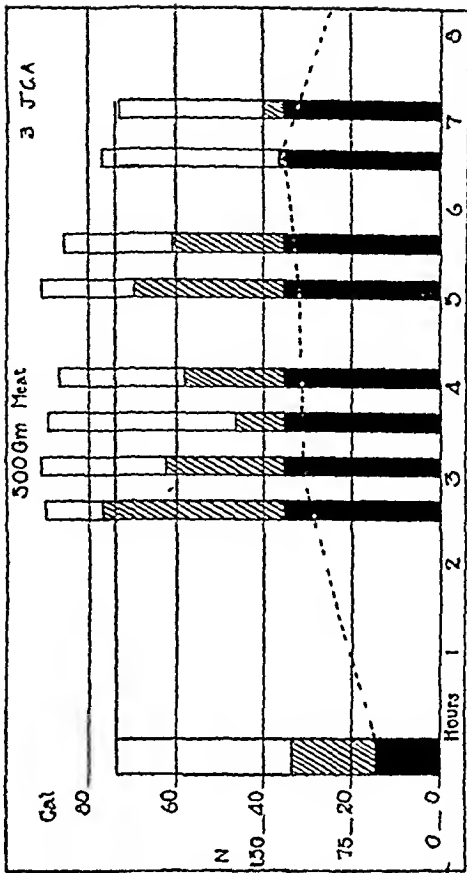
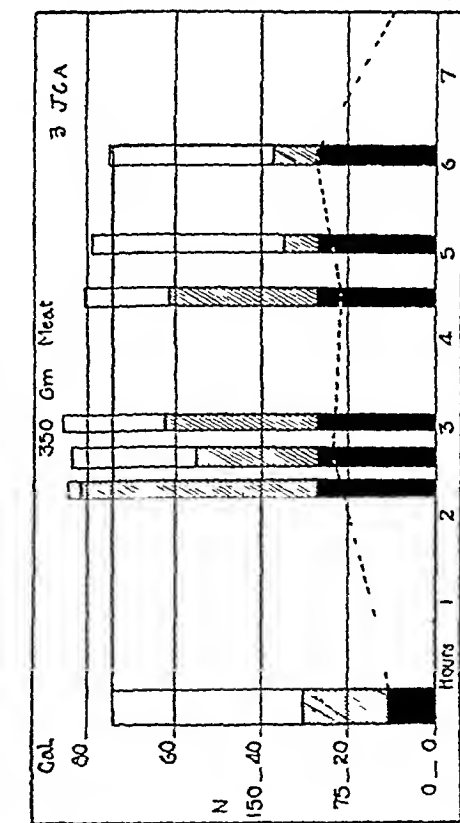
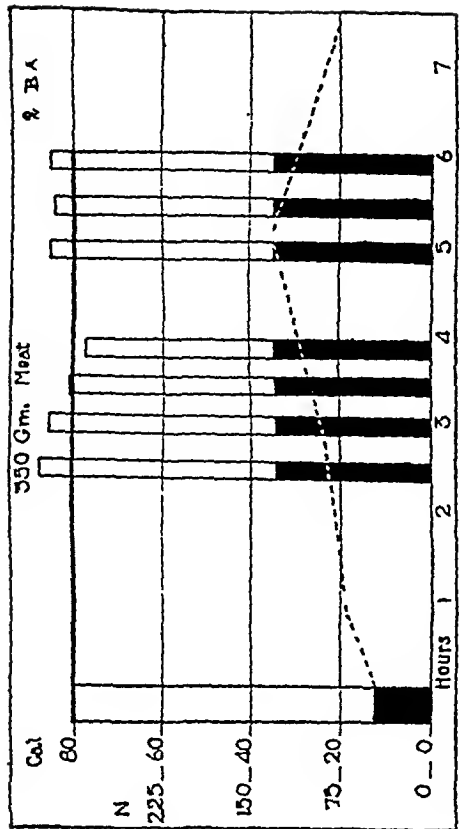
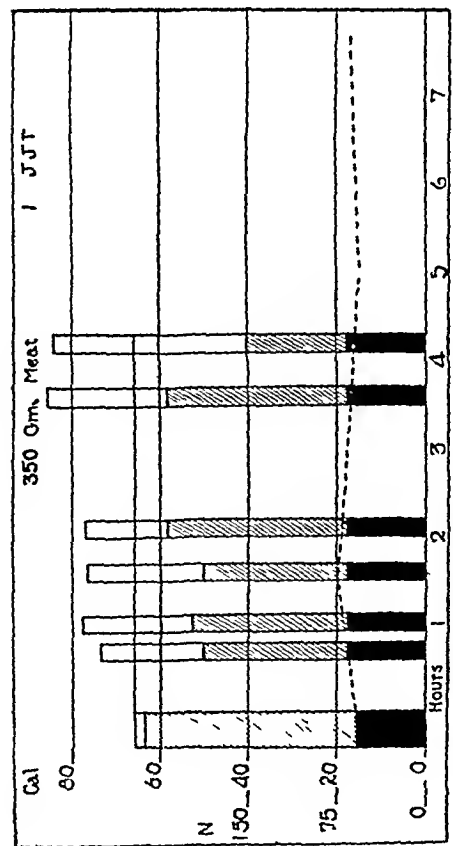


Fig 1—These records were made from four normal controls

The total height of the rectangles represents the total calories metabolized per hour. The solid black areas represent the calories of protein metabolized per hour, the hatched areas represent the carbohydrate and the unshaded areas represent the fat calories. The first observation is basal, those following are the observations taken after the ingestion of meat. The abscissa represents the time after starting the meat meal. The discontinuous line is the urinary nitrogen excretion in grams per hour. The points from which this is drawn represent the middle of the period of each urine accumulation.

acids in the urine and blood. These authors suggest that deamination and urea synthesis is incomplete without the liver, but may still occur to a considerable degree. Glaessner¹⁵ used abnormally high amino-acid nitrogen excretion as a liver function test, but his methods were criticized by Masuda,¹⁶ who did not get nearly as striking results in his tests. Ishihara¹⁷ produced chronic phosphorus poisoning in the dog and found no noteworthy change in amino-acid excretion as long as the animals would eat. Levene and Van Slyke¹⁸ found a normal amino-acid excretion in the urine of two cases of cirrhosis and in dogs poisoned with chloroform, and with chloroform and phosphorus. Marshall and Rowntree¹⁹ observed an increase in the nitrogen in the last stages of phosphorus and chloroform poisoning in dogs, but they²⁰ report only slight increases in amino-acid nitrogen in both urine and blood in cases of liver insufficiency. Work along these lines, therefore, has given varying results, but the distinct impression remains that the cleavage of amino-acids is not entirely normal, though striking changes are seen only in very severe liver insufficiency.

While urinary and blood studies, therefore, have given rather conflicting results, no one, so far as we know, has studied the response of the total metabolism to protein feeding in liver disease. This is the best method, in our opinion, for studying the beginning of the utilization of ingested protein, for Lusk² and Csonka²¹ have shown that the rise in heat production approximately parallels the speed of amino-acid metabolism, and Janney,²² has shown that the rate of protein metabolism seems practically identical with that of amino-acids. In undertaking our experiments, therefore, it seemed reasonable that if there is a delay or an abnormal response in protein metabolism, it might be demonstrated by the respiratory exchange. Human subjects with advanced liver disease, with portal obstruction, and obstruction in the bile passages were studied. No cases of acute yellow atrophy or of

15 Glaessner, K. Functionelle Prüfung der normalen und pathologischen Leber, *Ztschr f exper Path u Therapie* **4** 336, 1907.

16 Masuda, N. Ueber die Ausscheidung verfütterter Aminosäuren bei Leber und Stoffwechselkrankheiten, *Ztschr f exper Path u Ther* **8** 629, 1910.

17 Ishihara, H. Ueber die Stickstoffverteilung im Hundeharne bei subchronischer Phosphorvergiftung, *Biochem Ztschr* **41** 315, 1912.

18 Levene, P. A., and Van Slyke, D. D. Gasometric Determination of Free and Conjugated Amino-Acids in the Urine, *J Biol Chem* **12** 301, 1912.

19 Marshall, E. K., and Rowntree, L. G. Studies in Liver and Kidney Function in Experimental Phosphorus and Chloroform Poisoning, *J Exper Med* **22** 333, 1915.

20 Chesney, A. M., Marshall E. K. and Rowntree, L. G. Studies in Liver Function, *J A M A* **63** 1533 (Oct 31) 1914.

21 Csonka, F. A. Animal Calorimetry. The Rate at Which Ingested Glycocoll and Alanine Are Metabolized, *J Biol Chem* **20** 539, 1915.

22 Janney, N. W. A Note on the Rate of Metabolism of Proteins and Amino-Acids, *J Biol Chem* **22** 191, 1915.

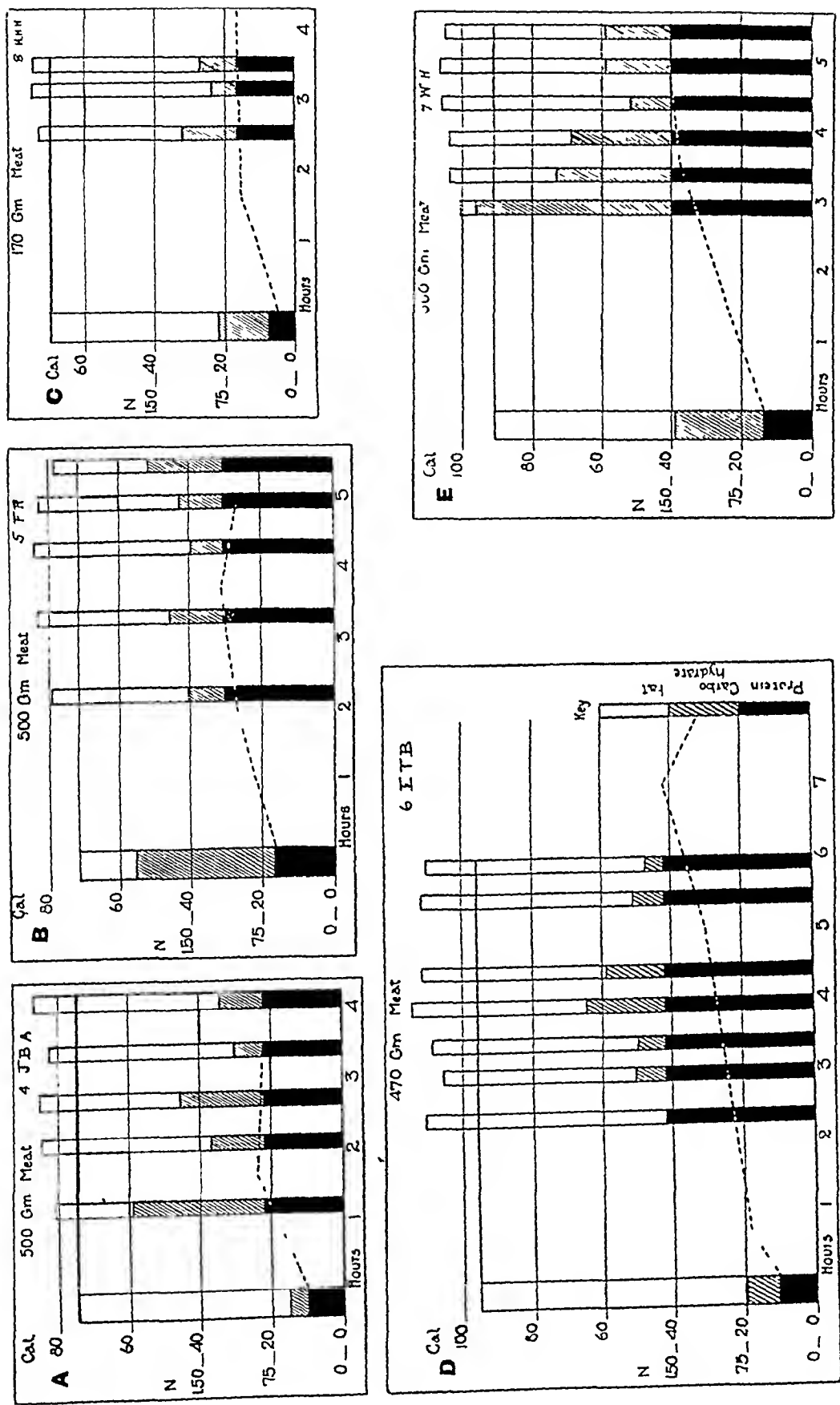


Fig 2—A and B are records made from normal controls C was a case of carcinoma probably involving the liver D and E were cases of exophthalmic goiter

phosphorus poisoning could be investigated. This is to be regretted, for these types of liver disease give the most striking changes in protein metabolism. Cases as uncomplicated as possible were chosen, and it was to be expected that the ability to utilize, as well as the speed of utilization, of protein foods could thus be studied. Mueller and Schultz²³ have assumed that much of the protein absorbed when there is obstruction of the portal circulation is poured into the ascitic fluid and then reabsorbed into the blood stream by way of the peritoneum. If this is true, one would expect a delay in the appearance of the dynamic action, and a reduction of its intensity. The work here reported was undertaken to study the response to protein-rich meals by patients with severe liver disease, and the results have shown that they react in an essentially normal way.

METHODS

The total metabolism was studied with a Benedict universal respiration apparatus by the methods previously described in papers from this laboratory^{24, 25}. The data obtained included the oxygen absorbed and the carbon dioxide eliminated over experimental periods of about ten minutes duration. The basal figure is the average of repeated basal determinations made, usually, the day before the test meal, but the other figures in the charts are from single observations. Urinary nitrogen determinations were made by the Kjeldahl method. These analyses were made always in duplicate, usually in triplicate. The total calories used and the percentage of utilization of carbohydrate, fat and protein were calculated from the carbon dioxide and the oxygen exchange, and the nitrogen eliminated in the urine²⁵. All figures and calculations have been checked carefully.

Because of the delay in nitrogen elimination, as discussed in a paper by Aub and DuBois,⁴ it seemed wiser to take the maximum rate of urinary nitrogen elimination as the index of protein metabolism after meat ingestion. This was done because there is much evidence to show that the normal stimulation of metabolism by protein is at its height within two hours after eating, but that the maximum nitrogen excretion

23 Muller, L. R., and Schultz, O. Klinische, physiologische, und pathologisch-anatomische Untersuchungen an einem Fall von hochgradigem Ascites bei Pfortaderthrombose, Deutsch, Arch. f. klin. med. **76** 544, 1903.

24 Means, J. H. Studies on the Basal Metabolism in Obesity and Pituitary Disease, J. M. Research **27** 121, 1915.

The case of W. K. was studied by the Tissot method at the Peter Bent Brigham Hospital. We wish to take this occasion to thank Dr. Cyrus Sturgis and Miss Edna Tompkins for making the respiratory determinations on this patient for us, and Dr. Reginald Fitz for the chemical analyses.

25 Lusk, G. A Respiration Calorimeter for the Study of Disease, Arch. Int. Med. **15** 793 (June) 1915.

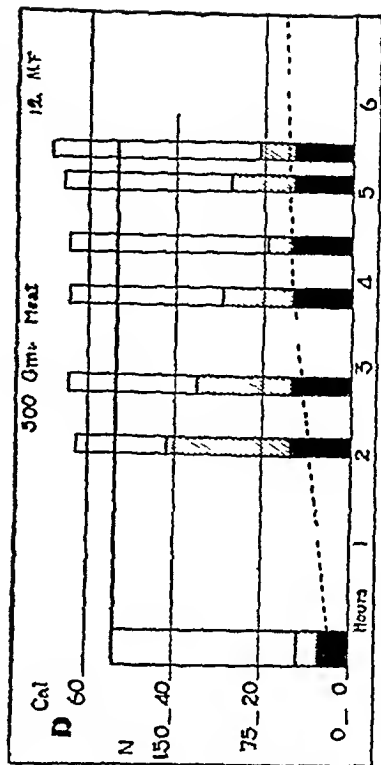
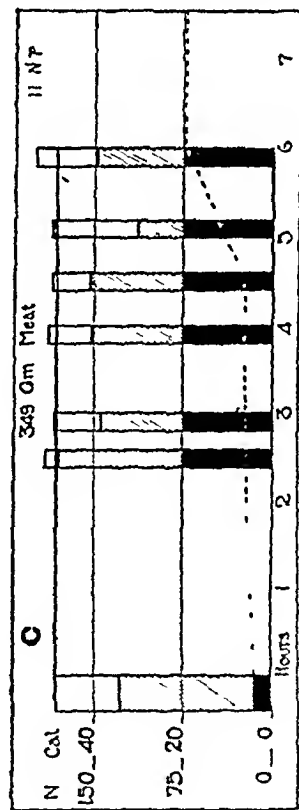
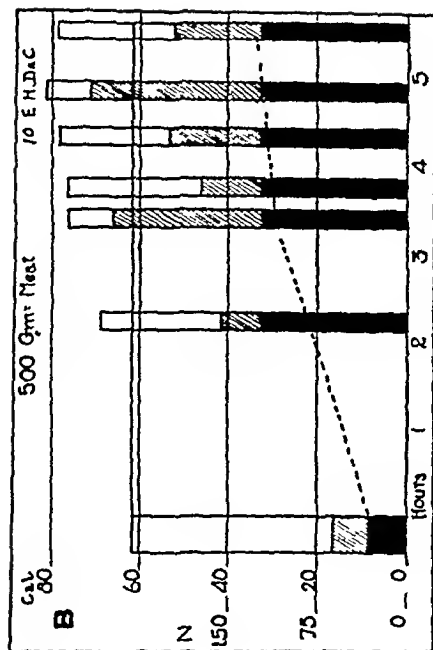
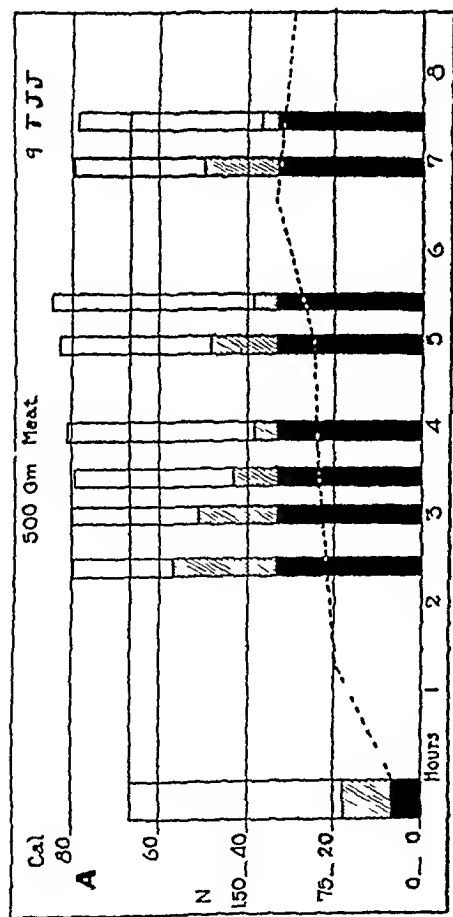


Fig 3—A and B are records made of two cases of obstructive jaundice C was a case of gallstones D was a case of cirrhosis with obstruction in which ten hours and forty minutes after eating the nitrogen curve was 0.672 gm per hour.

may not occur for six hours after the meal ²⁶ When compared with the cases of Aub and DuBois, the nitrogen and the sulphur excretion in the cases of liver disease did not differ from the normal response

With one exception, the subjects all ate, approximately either 350 or 500 gms lean beefsteak, separated from all fat and gristle The steak was finely chopped and cooked as meat balls, without butter With it was served a very little stewed tomato, and water Gigon ²⁷ claimed that the specific action did not vary directly with the amount of protein eaten The normal controls, however, give a direct basis of comparison for the ingestion of either 350 or 500 gms meat

The actual figures of our experimental data are not given in this paper, because of their very large bulk, and because we feel that the graphic presentation of the experiments while more compact is of equal value The summary tables, however, are given

HISTORIES

The histories of the cases studied are here given very briefly In the hospital they were studied with great thoroughness, but only positive and pertinent findings are here recorded

Control 1—J J T (W S 214,249²⁸) was a young man, aged 24 years, who had had an operation for inguinal hernia ten days before study His convalescence had been uneventful, and we, therefore, considered him normal, except that he had been bedridden

The other controls (Nos 2, 3, 4 and 5) were doctors and medical students working in the hospital They were all normal men and in fair physical condition

Two exophthalmic goiter patients were studied in relation to other work

CASE 1—(6) E T B (E M and W S 213,101), aged 35 years, was a man with moderately severe exophthalmic goiter of six years' duration His case is reported fully by Means and Aub²⁹ as Case 137, and by Means³⁰ as that of Mr E T B

CASE 2 (7)—W H (W M and W S 210,852), aged 22 years, was a man with exophthalmic goiter who was still very toxic in spite of a ligation of the superior thyroid arteries done at the Mayo Clinic This is Case 83 of Means and Aub He was operated on after the tests that are reported here, and one month after removal of a large part of the thyroid his metabolism was normal

Cases of Liver Disease—All of these patients were men except Nos 11 and 12

²⁶ Williams, H B, Riche, J A and Lusk, G The Metabolism of the Dog Following the Ingestion of Meat in Large Quantity J Biol Chem **12** 349 1912

²⁷ Gigon, A Ueber den Einfluss der Nahrungsaufnahme auf den Gaswechsel, Arch f physiol **140** 544, 1911

²⁸ All numbers following the initials of patients refer to the Massachusetts General Hospital records except Case 16, who was a patient at the Peter Bent Brigham Hospital

²⁹ Means, J H, and Aub, J C The Basal Metabolism in Exophthalmic Goiter, Arch Int Med **24** 645 (May) 1919

³⁰ Means, J H M Clin N America 1099 (January) 1920

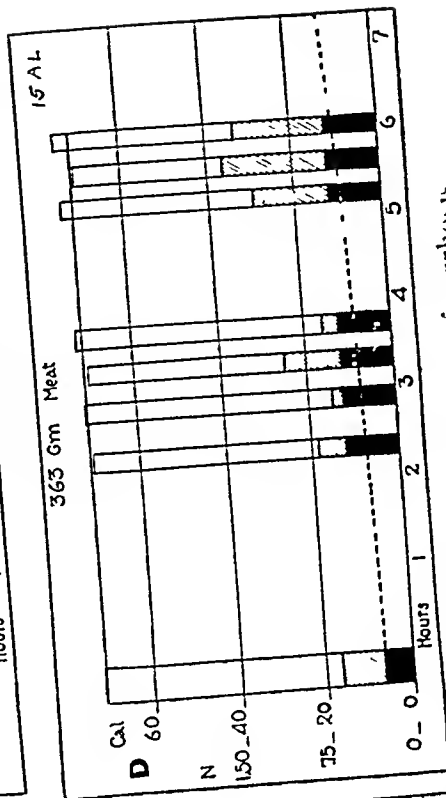
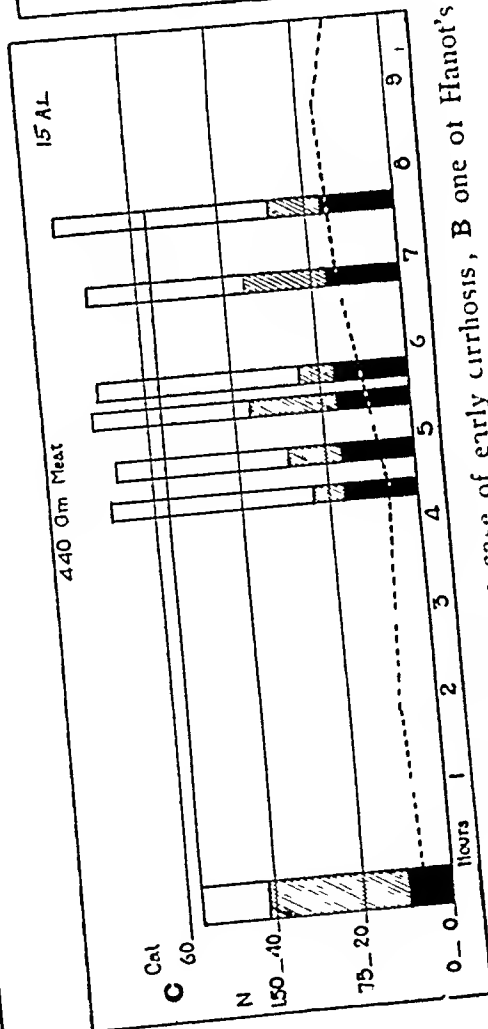
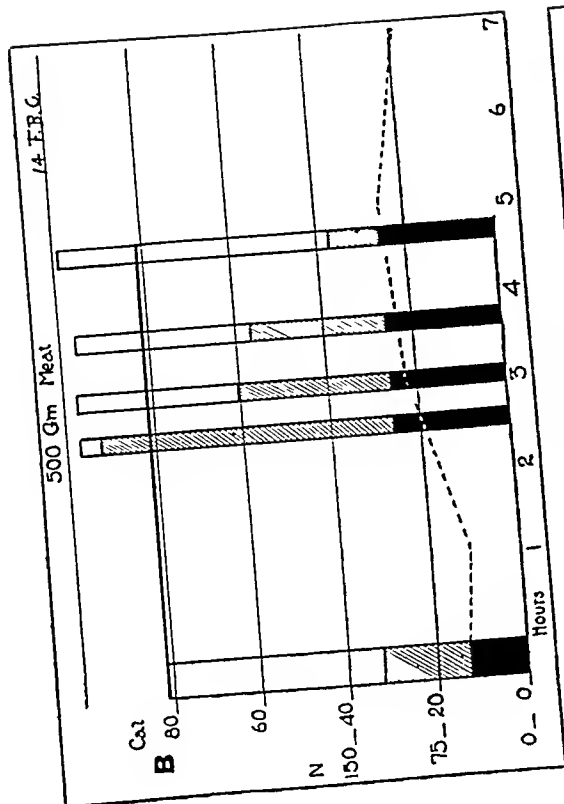
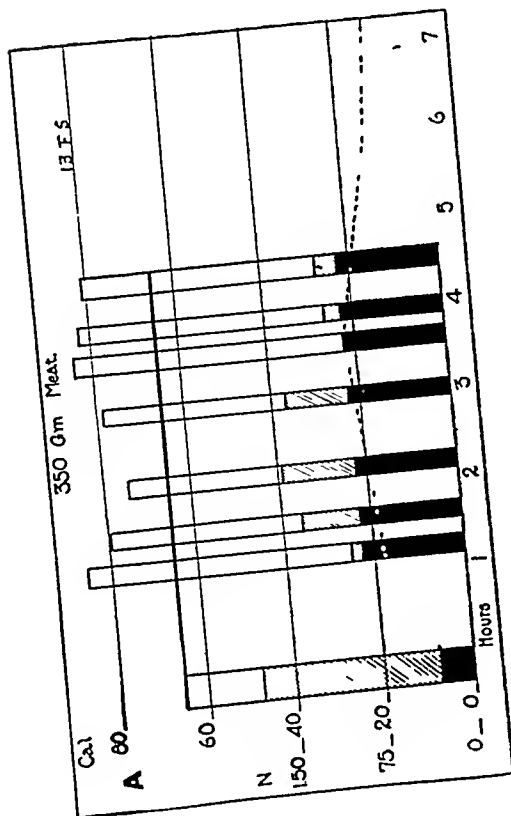


Fig 4—A was a case of early cirrhosis, B one of Hanot's cirrhosis, C and D was a case of cirrhosis

CASE 3 (8)—H H H (E M 210,905) was a married negro, aged 42 His occupation was that of restaurant chef

For the past seven years, he had had occasional gastro-intestinal upsets with gnawing epigastric pain and nausea every few months after indiscretions in diet They were relieved by vomiting and sodium bicarbonate Five weeks previously these had become very frequent Three weeks previously the pain had shifted to the right side, and a constant dull ache remained which extended into the back and occurred with deep inspiration There were then no more gastro-intestinal symptoms Bowels had been regular and the urine normal. He said that he had lost 13 pounds weight in the last six months He had never been a heavy drinker

The physical examination was negative, except for marked pyorrhea The left pupil was larger than the right, both pupils were slightly irregular Liver dulness extended from the fifth rib to $6\frac{1}{2}$ cm below the costal margin The edge was sharp and tender

Laboratory Findings—Roentgen-ray examination showed no positive evidence of gallstones Urinary examination showed a very slight trace of albumin, and red blood cells, a few leukocytes, and no casts in the sediment Thirty-five per cent phenolsulphonephthalein was excreted in two hours No autolysis of the blood clot The Wassermann test was strongly positive The blood pressure was 115/70 In the specific dynamic action test, the patient could eat only 170 gm meat, and felt that this distressed him

He left the hospital unoperated, with the diagnosis of syphilis of the liver He died three weeks later His rapid death suggests a new growth rather than syphilis of the liver No necropsy was obtained, so that the diagnosis is somewhat in doubt

CASE 4 (9)—T J J (E M and E S 213,698) was a cabinet maker, aged 51, married, who had drunk ten glasses of ale a day as well as two or three brandies a week for years Four months before entry he had had a sharp pain on the right side of the epigastrium, radiating to the midline, which was so severe that it doubled him up Two days later he became jaundiced Since that time he had lost 20 pounds in weight

Physical examination showed that the patient was very jaundiced, the liver was easily palpable 4 cm below the costal margin, with a rounded edge, and a smooth, not tender surface

Laboratory Findings—Bleeding time, three minutes No autolysis of blood clot in twenty-four hours The stools showed a positive guaiac test twice The gastric test meal gave a negative guaiac test Urinary examination showed no albumin, bile ++, and many casts in the sediment Blood pressure, 100/62

Following our studies an operation was performed A hard, scirrhus type of cancer was found at the junction of the cystic and common bile ducts, and there was marked backing up of thick bile The duct and gallbladder were drained

Diagnosis—Carcinoma of the bile passages

CASE 5 (10)—E H DeC (E M 211,730) was a weaver, aged 29 years, who until shortly before entry had drunk three or four glasses of gin and from six to seven glasses of beer daily, and excessively every Saturday night Eleven weeks before entry, a pain started in the upper abdomen which was severe at first, but which became gradually sharper and constant For ten weeks he had had gradually increasing jaundice with six clay colored stools a day He had lost 20 pounds in weight and was growing rapidly weaker, in spite of an excellent appetite

Physical examination showed a very poorly nourished but well developed man, who was markedly jaundiced His heart rate was 44 per minute Liver dulness extended from the fourth intercostal space to $2\frac{1}{2}$ cm below the costal margin The edge was easily felt, and was hard, smooth, slightly rounded and

not tender The gallbladder was also palpable The patient's weight fell in one week from 108 pounds to 99 pounds while he was in the hospital

Laboratory Findings—Roentgen-ray examination of the gallbladder and gastro-intestinal tract showed no definite lesion Cardiac study showed brachycardia to be the only abnormality Urinary examination showed the slightest possible trace of albumin, bile + + + + in two examinations, and a negative sediment The stools showed a guaiac test + + + in four examinations Vomitus gave a positive guaiac test Blood pressure, 105/50

Subsequent History—After leaving the Massachusetts General Hospital, the patient was operated on at the Lawrence Hospital The operation showed sarcoma of the pancreas and obstruction of the bile duct Sarcomatous nodules later appeared on the body The patient died at the end of January, 1917

CASE 6 (11)—N P (E M and E S 311,013) a married housewife, aged 53 years, had her menopause two years before entry Ten years before entry she had had a typical gallstone colic which had recurred several times since then, but never with jaundice Since a typical attack six weeks before entry, she had had dull pain

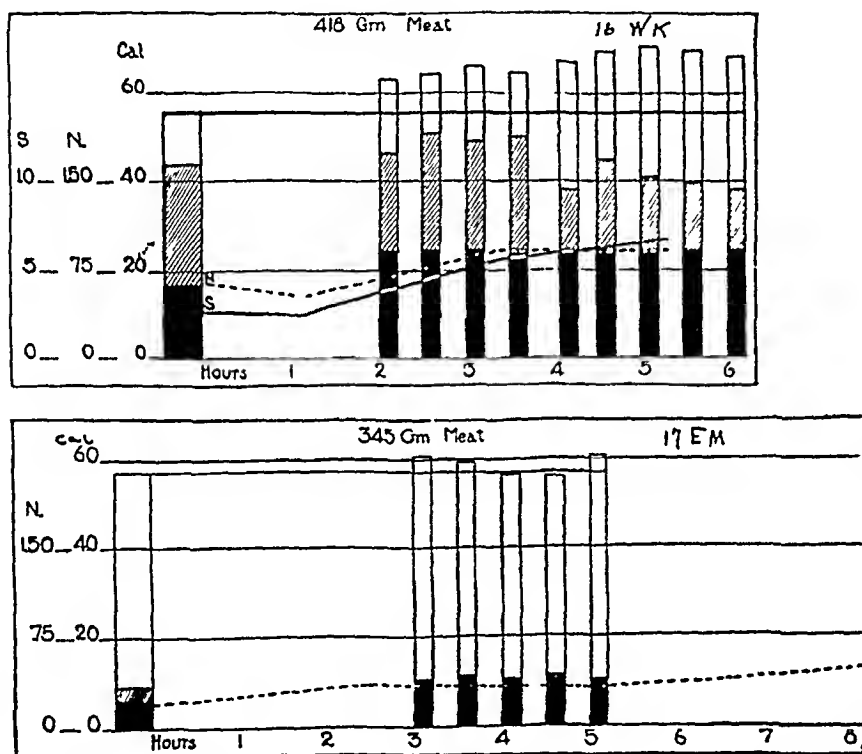


Fig 5—Both records were made of cases of cirrhosis In the upper record, the curved continuous line is the sulphur excretion per hour

Physical examination showed nothing remarkable, except that liver dulness extended from the fifth rib to $5\frac{1}{2}$ cm below the costal margin The edge could be felt as slightly tender and sharp

Laboratory Findings—These were all negative, except for a positive guaiac test in the gastric contents

Following our study Dr F G Balch operated and found a thickened gallbladder with two large stones The liver was large, dark colored, and felt granular The pylorus was thickened, but no definite lesion was found

Diagnosis—Gallstones

CASE 7 (12)—M F (E M and E S 212,732) was a housewife aged 28 years Three years before entry, after her last baby was born, she was

jaundiced for one week. Five weeks later, she had a sharp pain in mid epigastrium, with nausea, vomiting and slight jaundice. These attacks recurred very frequently. One and one-half years before entry she was operated on, and a gallbladder filled with stones was removed. The wound was drained for five weeks before healing and from that time on, fluid accumulated gradually in the abdomen. For the past ten months her abdomen had been tapped every two or three weeks. She had no pain, felt well and continued to work.

Physical examination showed that there was a slight jaundice of the sclerae, that the abdomen was very full of fluid, that there was slight edema of the legs, and that there were a few internal hemorrhoids.

The patient was tapped three times in two weeks, 17 pints of fluid being obtained each time. After tapping, the liver edge could be felt as rounded, lobulated, and hard, slightly tender, and descending on respiration.

The laboratory findings were all negative, except that the blood pressure was 105/75, and that the stools showed a faintly positive guaiac test once in four observations. The ascitic fluid showed a specific gravity of 1.016 and 1.018, 160 cells per c mm, of which 54 per cent were epithelial, 32 per cent lymphocytes, and 14 per cent neutrophils. The chemical findings of the fluid were reported as Cases No 27 and 28, Table 1, by Denis and Minot³¹.

Following our studies, Dr Daniel Jones operated and found the liver very hard, not enlarged, lobulated, but not granular. It felt like the knuckles of the hand. The vessels of the portal system were enormously distended. There were many adhesions all over the liver and about the portal vein, and a very vascular omentopexy. The spleen was hard and large.

The patient was discharged unrelieved, with a diagnosis of cirrhosis of the liver. She died soon afterward.

While no corroboration of the diagnosis can be given, the case is very similar to those described by Hoover³² of obstruction of the portal vein.

CASE 8 (13)—F S (E M 214,524) was a meat cutter, aged 45 years. Since he was 18 years old he had drunk one or two quarts of gin, whiskey or brandy each day, and occasionally even more. Six years before entry his abdomen had become swollen, but returned to normal size without treatment. For the past three years he had been jaundiced after unusual drinking bouts. For the last year he had felt tired and had lost about 20 pounds in weight, and occasionally he had vomited. Two months before entry, he had been jaundiced for a short period. There had been no swelling of the abdomen.

Physical examination was negative, except that the liver dullness extended from the fifth rib to 4 cm below the costal margin, the edge was palpable.

Laboratory Findings—No autolysis of blood clot in forty-eight hours. Poorly digested food was found in the sediment of the stools. There was no blood present. The temperature varied between 98 and 99.5 F.

The case was considered an early cirrhosis of the liver, before severe symptoms developed.

CASE 9 (14)—F B C (W M 212,049) was an unmarried travelling salesman, 27 years. Four years before entry, he had had an attack of jaundice without other symptoms. He recovered from this in four weeks, but had always been more or less yellow ever since the attack. Two years before entry he had been acutely ill with chills, fever, malaise, jaundice and clay-colored stools, and was in a New York hospital for sixteen weeks. There he developed red, swollen joints and one knee had since that time been stiff and deformed. He had never had any other gastro-intestinal disturbances and had lost no weight.

31 Denis, W, and Minot, A S. The Nonprotein Constituents of Edema Fluids, *Arch Int Med* 20:879 (June) 1917.

32 Hoover, C F. Obstruction of the Hepatic Veins, *J A M A* 74:1753 (June 26) 1920.

Physical examination showed marked jaundice. The abdomen was full, tympanic and was held so rigidly that the liver edge could not be felt. Liver dulness extended from the third costal interspace to 2 cm below the costal margin. The right wrist and left knee had limited motion, said by an orthopedic consultant to be the result of infection.

Laboratory Findings—Roentgen-ray examination of the gallbladder gave no evidence of stone. Hypertrophic changes were found in the joints. The urine showed a small trace of albumin and bile +. Red blood cells were seen twice in the sediment, but a culture was negative. No autolysis of blood clot. The stools showed a positive guaiac test twice, a negative test once, bile was present. Microscopic examination showed excess of fatty acids and soaps.

Diagnosis (by Dr Roger I Lee)—Hanot's cirrhosis, apparently secondary to a localized infection in the biliary passages.

CASE 10 (15)—A L. (E M 179,058) was a married Italian laborer, aged 51 years, who had drunk four or five glasses of whiskey and three or four glasses of beer a day for twenty years. The patient had been in the hospital five years before with a diagnosis of advanced cirrhosis of the liver and acute infection. He then had to be tapped three times in twenty-five days. During that period at the hospital he was operated on and an omentopexy was performed. Since then he had been tapped frequently but the ascitic fluid continued to recur with increasing rapidity. The liver at the time of operation was found to be small and hobnail in character.

TABLE I—BASAL METABOLISM DATA OF NORMAL CONTROLS

Subject's Number and Initials	Height	Weight	Age	Total Calories per Hour	Percentage Variation from Normal Standards	
					DuBois Surface Area Formula	Harris Benedict Tables
1 J. T.	160	77.0	24	65.5	+4	+5
2 B. A.	178	67.3	26	80.3	+11	+13
3 J. C. A.	176	60.4	27	74.2	+7	+12
		79.7		73.8	+7	+12
4 J. B. A.	170	64.8	23	74.3	+7	+8
5 F. R.	187	73.2	30	71.3	-8	-5
Average deviation					+5	+8

Physical examination showed a very poorly nourished and jaundiced Italian with slight dyspnea whose teeth were in very poor condition. Heart measurements 6 cm to right of midsternal line, 15½ cm to left in the fourth interspace. The sounds were of good quality. The abdomen measured 116½ cm, the chest 88½ cm. There were signs of much fluid in the abdomen and the veins were markedly dilated. In the legs also the veins were dilated.

Laboratory Findings—Urinary examination was negative, except that bile was present. No autolysis of blood clot in twenty-four hours. The nonprotein nitrogen of the blood was 36 mg per 100 cc. The patient was tapped eleven times in seventy-five days and an average of 7 pints of fluid was obtained, which showed a specific gravity of about 1.015, about 40 cells per c mm, most of which were lymphocytes. Thorough chemical studies of the ascitic fluid are reported by Denis and Minot in Table 2 of their paper. These studies show that the concentration of urea, uric acid, and cholesterol in the fluid could be influenced by changes in diet.

Impression—A case of very severe alcoholic cirrhosis of long duration.

CASE 11 (16)—W. K. (Brigham Hospital, 22,290) was a laborer aged 65 years, who had acquired syphilis eleven years before entry. For the past two years he had had nocturia, from 2 to 4 times. All of his teeth had been extracted two years before, but he had never had false teeth. Two weeks

before entry be noticed that his abdomen was swollen. He had no pain and continued to work. He was easily tired, however, and had considerable coughing at night. Both legs seemed swollen at that time.

The patient showed considerable loss of weight, edema of the feet and ankles, and a marked ascites. Peripheral vessels showed marked arteriosclerosis.

The patient was tapped and 8 liters of ascitic fluid were obtained. The liver and spleen could not be felt.

Laboratory Findings—Roentgen-ray examination of the lungs showed adenopathy in hilus regions, and mottling of both lungs.

After tapping, the fluid did not recur, and the patient was discharged relieved, with a diagnosis of alcoholic cirrhosis of the liver, arteriosclerosis, ascites and question of pulmonary and peritoneal tuberculosis.

Impression—This case gave the impression of being a fairly severe cirrhosis with the first signs of liver insufficiency.

CASE 12 (17)—E M (E M and E S 210,942) was a married man, aged 54 years, with no occupation. He had drunk from one-half to one pint of gin and whiskey every day for twenty years, but for the past ten years he had drunk very little. A year before entry his abdomen had become large, but it diminished in size after rest and treatment. Three months before entry it again became swollen, and in the last two weeks had markedly increased in size. He had had edema of the legs for three months, and of the scrotum for four days, and had lost much weight. He had never been tapped.

Physical examination showed a very thin man. The sclerae were possibly slightly yellow. The arteries were sclerotic, and the brachials tortuous. The abdomen was full of fluid. The veins of the abdominal and chest walls were markedly dilated.

The patient was tapped and 16½ quarts of ascitic fluid were taken from the abdomen. The edge of the liver could not be felt after this. Six days later the abdomen was again filled as at entrance.

The patient was operated on by Dr E P Richardson. About 3 gallons of yellow, serous fluid were evacuated. The liver was contracted and hobnail in type. The spleen was twice its normal size. The stomach appeared normal. Omentopexy was performed.

Laboratory Findings—Examination of the blood showed a red cell count of 3,500,000, 80 per cent hemoglobin, moderate achromia, 35 mg nonprotein nitrogen per 100 cc blood. Autolysis of blood clot was negative once, but positive once in forty-eight hours. In the ascitic fluid there were 45 cells per cmm of which 60 per cent were lymphocytes and 36 per cent endothelial cells. The chemical analysis of the fluid is reported as Case 6 in Table 1 by Denis and Minot.

The patient died October 17. Necropsy (3646) showed chronic interstitial hepatitis, slight hypertrophy of spleen, ascites, slight icterus, chronic pleuritis, hemorrhagic edema of lungs, slight arteriosclerosis, operation wound, omentopexy.

CASE 13 (18)—S B (E M 210,639) was a married cobbler, aged 38 years, with moderate habits. Four weeks before entry, he had chills and fever for one day, with a cramplike constant pain which did not radiate, in the right hypochondrium. He had had no pain since then, but his legs had become swollen and the abdomen distended, and for two weeks he had been jaundiced.

Physical examination showed the patient to be slightly cyanotic. His abdomen was distended and there were signs of the presence of much fluid. There was marked edema of the legs and thighs. The heart was felt in the fourth interspace, 14 cm to the left of the midsternal line. Its sounds were regular and normal, except for a slight blowing systolic murmur at the apex, which was transmitted slightly to the axilla.

Laboratory Findings—Roentgen-ray examination showed the heart shadow to be within normal limits. Phenolsulphonephthalein test showed 30, 55, and 51 per cent respectively to be excreted in two hours. Twenty-seven mg nonprotein nitrogen per 100 cc were found in the blood. Two urinary examina-

tions showed the slightest possible trace of albumin. The blood pressure was 145 systolic.

Subsequent History—The patient was discharged, but reentered the hospital two weeks afterward because of recurring edema of the legs and abdomen. Dr. D. L. Edsall in a note in the record stated that he thought the case was one of polyserositis, and that the liver trouble was more important than the cardiac condition. When the patient was tapped Oct. 26, 7000 cc fluid were obtained. This was turbid, and had a specific gravity of 1.011. It contained 350 cells per cmm of which 62 per cent were small lymphocytes. A guinea-pig inoculated with the fluid developed tuberculosis of the spleen. The chemical analysis of the fluid is reported as Case 9, Table 1, in the paper by Denis and Minot.

October 30 the patient grew irrational, his intestines were dilated and there were signs of fluid in the pericardium. He died the same day. A necropsy was refused.

Final Diagnosis—Cardiac decompensation (slight), and question of cirrhosis of the liver.

TABLE 2—BASAL METABOLISM DATA IN LIVER DISEASE

Patient's Number and Initials	Height	Weight	Age	Temp., F	Total Calories per Hour	Percentage Variation from Normal Standards	
						DuBois Surface Area Formula	Harris Benedict Tables
8 H. H. H.	170.0	62.5	42	97.6	70.4	+6	+13
9 T. J. J.	167.2	78.2	31	98.2	66.1	+7	+17
10 F. H. Dec.	163.0	46.0	29	98.6	61.7	+7	+12
11 N. P.	143.5	33.0	53	97.6	48.6	-3	-1
12 M. I.	154.3	54.1	28	98.0	53.8	-4	-3
13 F. S.	175.0	58.6	45	99.4	65.5	±0	+9
14 F. B. C.	189.5	73.3	27	97.0	81.2	+7	+8
15 A. I.	163.5	63.4	51	98.4	71.0	-11	-4
	163.5	64.2	51	99.6	56.4	+11	+20
16 W. K.	169.0	71.9	65	97.3	55.5	+1	+12
17 J. M.	177.5	68.6	54	97.8	57.1	-18	-11
18 S. B.	164.0	81.4	38		75.2	±0	+1
19 F. P.	170.0	56.8	22	97.2	71.5	+13	+15
Average deviation						+1	+7

* 1 female

Diagnosis at Death—Multiple serositis, ascites, left hydrothorax.

CASE 14 (19)—F. P. (W. M. 213,436) was an unmarried butcher, aged 22 years. Fifteen months before he was operated on for gangrenous appendix. Three months later two ribs, a portion of the abdominal wall, and a wedge of the liver were removed, because of actinomycosis. At the time of this study he had a typical case of acute catarrhal jaundice, of one month's duration, from which he was beginning to recover.

Diagnosis—Acute catarrhal jaundice.

DISCUSSION

The Basal Metabolism—Several publications by Grafe³³ showed that in animals with Eck fistula with the circulation of the liver cut off [1]³³ or whose liver had been removed, [2]³³ the total metabolism was

33 (1) Grafe, E. and Fischer, F. Der Einfluss der Leberausschaltung auf den respiratorischen Stoffwechsel. *Deutsch Arch f klin Med* 108:516, 1912. Das Verhalten des Gesamtstoffwechsel bei Tieren mit Eck'scher Fistel 104:28, 1911. (2) Grafe, E., and Deneke, G. Ueber den Einfluss der Leberextirpation auf Temperatur und respiratorischen Gaswechsel, *Deutsch Arch f klin Med* 118:249, 1915.

reduced as much as one third. With this drop in metabolism, however, there was a distinct fall in temperature which alone might cause a similar drop in heat production. The animals with the liver removed lived at most only fifteen hours. If the liver is essential to a normal basal metabolism, then very severe liver disease might show abnormal values.

In Tables 1 and 2 are summarized our data for basal metabolism. The usual procedure was followed in all these cases—the subjects had not eaten for fifteen or eighteen hours, and the tests were made only after complete muscular relaxation of more than half an hour's duration. The twelve cases of liver disease are described in the case histories. The patients all had distinct involvement of the liver, most of them with very advanced disease and severe symptoms. They were chosen largely because of their varied types of disorder. It was thought best to report the variations by both the DuBois³⁴ and the Harris-Benedict³⁵ standards, but the average by both methods is well within the normal limits. By the DuBois method, only two cases are really outside normal limits, and this may be explained by factors other than the liver. E. M. was very emaciated and had far advanced cirrhosis—a man who, because of his emaciation alone, should have had a reduced metabolism. F. P., a nervous, high-strung young man, should normally have had a slightly elevated metabolic rate. Therefore, it seems justifiable to conclude that the basal metabolic rate in liver disease is normal, and that either the liver is not an important regulator of the metabolic rate, or that, even in severe disease, it is adequate to accomplish this function.

Response to a Protein-Rich Meal—The reactions of metabolism after the ingestion of large quantities of meat are best shown in Tables 3 and 4, and in the charts in which are graphically portrayed the height of metabolism and the duration of the experiment. It is readily seen that in all but three cases, the response to protein food is quite similar to that in the normal individual. The elevation of metabolism occurs as rapidly as in normal cases when there is portal obstruction with rapidly recurring ascites, and also with very severe liver disease, with practically complete bile duct obstruction. Tests in two cirrhosis cases and in one case of gallstones failed to show any specific rise of metabolism. As there was no marked rise in the urinary output of nitrogen, it may be assumed either that the protein was not being

34 (1) Gephart, F. C., and DuBois, E. F. The Basal Metabolism of Normal Adults with Special Reference to Surface Area. *Arch. Int. Med.* **17** 902 (June) 1916. (2) Aub, J. C., and DuBois, E. F. The Basal Metabolism of Old Men, *Arch. Int. Med.* **19** 831 (June) 1917.

35 Harris, J. A., and Benedict, F. G. *Biometric Standards*. Publication No. 279, Carnegie Inst. Washington.

TABLE 3.—SPECIFIC DYNAMIC ACTION IN NORMAL CONTROLS AND LOPHTHAIIMIC GOITER

[illegible]

TABLE 4—SPECIFIC DYNAMIC ACTION IN LIVER DISEASE

[illegible]

absorbed, or that possibly, which is unlikely, it was being deposited. One of these patients (A L) later showed a quite normal response, and so it is very likely that these three cases were due to faulty absorption resulting from the venous congestion of cirrhosis. It is difficult to explain any lack of absorption of protein as being due to gallstones. It is of interest that A L, who showed no markedly increased nitrogen excretion or specific dynamic action after his first test meal, did show a distinct increase in the protein content of the ascitic fluid after the meal. This suggests that part of the protein escaped into the peritoneal cavity to be reabsorbed, as Muller and Schultz believe²³. In order to catch a

TABLE 5—CALORIC INCREASE PER HOUR AFTER MEAL TEST

Patient's Number and Initials	Protein Calories			Total Calories Average			Ratio of Total Increase to Protein Increase
	Basal	Maximal	Increase	Basal	Maximal	Increase	
Controls							
1 J J T	15 48	17 05	1 57	65 47	84 00	19 43	12 31
2 B A	12 09	34 15	22 06	80 32	83 99	3 67	0 17
3 J C A	14 82	35 63	20 86	73 84	89 02	15 18	0 73
	10 07	26 72	16 65	74 17	84 45	10 28	0 62
4 J B A	10 28	22 37	12 11	74 33	89 99	10 66	0 83
5 F R	16 06	30 83	14 77	71 31	83 44	12 13	0 82
Exophthalmic Goiter							
6 E T B	10 66	42 09	31 43	96 09	110 45	14 36	0 46
7 W H	13 57	40 24	26 67	90 63	105 09	14 41	0 54
Liver Disease							
8 H H H	6 95	16 62	9 67	70 41	74 72	4 31	0 45
9 T J J	6 63	33 14	26 51	66 10	81 09	14 99	0 57
10 E H DeC	8 51	32 61	24 10	61 73	78 51	16 78	0 70
11 N P *	3 82	19 59	15 77	48 45	53 77	5 32	0 34
12 M F *	6 50	13 02	6 52	53 84	65 16	11 32	1 73
13 F S	7 62	22 77	15 15	65 49	83 48	17 99	1 19
14 F B C	12 25	25 61	13 36	81 45	97 55	16 10	1 20
15 A L	6 15	12 72	5 57	71 06	71 96	0 90	0 16
	9 83	16 73	6 90	56 42	71 72	15 30	2 21
16 W K	16 35	24 26	7 91	55 51	69 50	13 99	1 76
17 E M	5 14	10 23	5 09	57 12	58 91	1 79	0 35

* Females

possibly delayed response this test was repeated, and four and one-half hours after the test meal a definite specific dynamic action was found. In this case, therefore, a delayed response is suggested. In all the jaundiced cases, the dynamic action is quite normal. This agrees with the findings of Glaessner¹⁵.

It is very difficult to find a satisfactory way of comparing the intensities of the dynamic action. The most logical method seems to be the ratio $\frac{\text{Total calories increase}}{\text{Protein calories increase}}$, for this should show the total response for each extra calorie of protein metabolism. This ratio gives similar response in all cases (Table 5) except in one normal control, J J T, where the response is far too great for the protein increase. In the remaining tests, the highest ratios are in the cases of cirrhosis. The two cirrhosis tests with low ratios showed practically no response to

the protein meal and therefore can hardly be counted in this regard. The other five tests suggest that in cirrhosis the dynamic action is high.

The partition of foodstuffs utilized is not very clear-cut, and varies markedly in the different experiments. On the whole, however, the grams of carbohydrate utilized are increased at the expense of fat during the specific dynamic action. The increase in protein calories is in general greater than the increase in total calories (Table 5). Our results are calculated to give protein the greatest possible proportions in the metabolism and in spite of this, it must be concluded that, though the protein increase is enough to account for all the rise of metabolism, it is aided by an increase of carbohydrate, and a diminution in the burning of fat. This agrees with the findings of Aub and DuBois⁴ but disagrees somewhat with Gigon,²⁷ who thought that all the extra calories came from protein.

The two cases of exophthalmic goiter which we have studied have given results which agree with the findings of DuBois.⁵ In spite of their high basal rate, the response to a large quantity of meat was an increase of metabolism as rapid and intense as in the normal individual.

CONCLUSIONS

- 1 The basal metabolism in twelve cases of liver disease was essentially within normal limits. The liver is, therefore, either not an important regulator of the metabolic rate, or it is adequate for this purpose even when severely diseased.

- 2 The rate of absorption and utilization of protein in large quantities was usually normal, even in severe cirrhosis. In two cases of cirrhosis and one of gall stones the utilization of the protein was delayed or absent. Marked portal obstruction caused no delay in the appearance of the specific dynamic action of protein.

- 3 The cases of cirrhosis showed, on the whole, the highest metabolic response to protein catabolism.

- 4 The conclusion seems justified that either the liver is not the main site of the specific dynamic action of protein, or that it can adequately perform that function even in disease.

- 5 The specific dynamic action of protein results from an increased combustion of protein and carbohydrate, rather than of fat.

- 6 The observation of DuBois that in exophthalmic goiter a normal increase in heat production, due to protein, is superimposed on the high basal rate is confirmed.

THE DIAGNOSIS OF "EVENTRATION" OF THE DIAPHRAGM

WITH REPORT OF A CASE OF APLASIA OF THE RIGHT LUNG
AND RIGHT HALF OF THE DIAPHRAGM, ASSOCIATED
WITH CONGENITAL DEXTROCARDIA ¹

HORACE MARSHALL KORN, M D
CLEVELAND

I INTRODUCTION

So-called "eventration" of the diaphragm is understood to mean a high position of one-half of the phrenic leaf, conditioned, not simply on displacement, but on aplasia (congenital) or atrophy (acquired) of the muscle fibers of that half of the diaphragm. Obviously, the term is a misnomer, but it appears that the various terms which have been offered as substitutes, while in themselves more exact, have served by their multiplicity merely to add to the confusion in terminology. One is tempted to choose from among these terms one more suitable, but inasmuch as the condition is definitely defined, it may as well go by the name of "eventration" as by any other.

I propose in this paper (1) to make a critical review of the literature on eventration of the diaphragm principally from the standpoint of the diagnosis, attempting also to establish or disprove the authenticity of the reported cases, and (2) to lay emphasis on the necessity for a careful study of the respiratory excursion of the thorax in the endeavor to differentiate eventration of the diaphragm from the condition with which it has been invariably confounded—hernia of the diaphragm, and (3) to present two cases illustrative of the points of differentiation.

2 REVIEW OF THE LITERATURE

If one undertakes a careful search of original sources in this subject, he soon discovers many errors which have crept into subsequent writings, there to be perpetuated and magnified. Some of these I shall attempt to correct.

The first authentic case of eventration of which we have record is that of Jean Louis Petit,¹ the description appearing originally in his posthumous "*Traite des maladies chirurgicales, etc.*" the first edition of which, edited by Lesne, was published in 1774. Petit himself, however, does not in any part of his writings employ the term "eventration," but describes his case as a peculiar variety of diaphragmatic hernia.

* From the Medical Clinic of Lakeside Hospital.

¹ Petit. *Traite des maladies chirurgicales*. Paris 2 266, 1774.

It was not until the time of Cruveilhier² that the essential difference between hernia and eventration was elucidated. The credit for the introduction of the term "eventration" has been distributed variously. Part of this confusion is, no doubt, due to the fact that Cruveilhier directly contradicted himself in this connection. In the *Anatomie pathologique du corps humain* (1829), he states unequivocally that Petit's case had been shown by Beclard to belong more properly in the class of eventrations. Twenty years later, in his *Traité d'Anatomie pathologique générale*, he says it was Petit who gave the name of "eventration." Inasmuch as no reference to the term is to be found in Petit's original writings, one is inclined to accept Cruveilhier's original statement, crediting Beclard with being the first to make use of the term in critical differentiation of the condition from hernia of the diaphragm. In this connection it should be stated that Cruveilhier's first recorded reference to the condition of eventration, according to Betchov³ occurred in 1816. Petit's description of his case is very convincing. Clinically, the patient

'had been for a long time afflicted with a so-called asthma which to all appearances had no other cause than the hernia inasmuch as he felt himself relieved of the so-called asthma as soon as he had eaten.'

The patient having died of peritonitis, Petit says of the necropsy

As those who believed him asthmatic had mistaken the peritonitis for a pleural effusion, I commenced the section with the chest, where I found nothing but a little fluid with a tumor the size of a small gourd. The tumor was almost as large at the base as at the middle and terminated in a blunt cone having a height of 3 or 4 inches."

Section of the abdomen revealed the peritonitis and

"the parts contained in the hernia were all stuck together and adherent to the sac. As for the sac I discovered that it was nothing but a prolongation of the peritoneum, the diaphragm, and the pleura, together, without the slightest rupture of the membranes, or any opening in the muscular or tendinous fibers of the diaphragm."

Previous to Petit's case, there is but one which might be called into question. It is that of Senac (1729) considered by Bowditch⁴ in 1853 to have been in all probability a genuine instance of eventration. Reference to Senac's original article⁵ discloses what was more probably a case of right-sided interstitial pneumonitis and chronic fibrous pleurisy, with fixation of the diaphragm in a high position by pleural retraction and synechia. There is no definite statement about the condition of the pleura, the pleural sinus, or the musculature of the diaphragm.

2 Cruveilhier. *Anatomie pathologique du corps humain*, Paris, 1829, Tome I, Livr. XVII, *Traité d'Anatomie pathologique générale*, Paris, 1849, Tome I, pp. 614 and 617.

3 Betchov. *Rev. méd. de la Suisse Rom.* **37** 455, 1917.

4 Bowditch. *Buffalo M. J.* **9** 1, 65, 1853.

5 Senac. *Sur le diaphragme*. (From *Mem. Acad. roy. d. sc. de Par.*, 1729.) *Collect. Acad. d. Mem.*, etc., Par. **6** 440, 1781.

In 1783, Pyl⁶ described a case which, according to Lacher,⁷ showed the left side of the diaphragm to be attenuated and dilated like a hernial sac. Glassner,⁸ and many others, have likewise been convinced of the genuine nature of this case. Access to the original article was denied me, but in view of Lacher's misconception of the cases of Froriep and Meckel one cannot but be somewhat skeptical of his interpretation.

It is when one comes to the consideration of the cases described by Meckel and Froriep that the more conspicuous instances of perpetuation of error appear. These cases have been generally accredited as true examples of eventration since they were first so labelled by Lacher in 1880. To quote from Lacher directly,

In Kleinert's Repertorium, 1837, a case of Froriep's is cited, in which at the necropsy of a 19 year old girl who had complained constantly of shortness of breath, vomiting and other gastro-intestinal disturbances, and since her fourteenth year had been troubled with obstinate constipation, the left diaphragm was found to be an upward-projecting sac containing the stomach, displacing the heart considerably to the right, the diaphragm was abnormally thin and in the region of the mediastinum changed to a transparent cellular tissue. This abnormal thinning out seemed to be definitely congenital, for no other cause, as for instance, inflammation of the diaphragm or diaphragmatic pleura, was mentioned.

A careful search of all four volumes of the Repertorium for 1837 reveals but one reference to such a condition by Froriep,⁹ which is herewith set down verbatim.

A diaphragmatic hernia where the stomach lay in the left half of the chest, pushing the heart toward the right was found in the corpse of a girl of 19 years, who from childhood had suffered from dyspnea, vomiting and other gastro-intestinal disturbances.

The identity of the two subjects seems quite clear, but the disparity in the reports is too obvious to pass unchallenged.

Of Meckel's case, Lacher says that on the left side the diaphragm was arched out like a sac. The description of this case in Zwanziger's original article¹⁰ (1819) is as follows:

A mature feminine fetus. The left pleural sac contains, besides the normal (1) the stomach, with (2) the small intestine, except the duodenum, (3) the greater part of the colon, (4) the spleen. The parts are thus arranged. The esophagus, descending in the usual manner to the vicinity of the diaphragm, is turned a little bit upward and enters the stomach. The stomach is turned in a

6 Pyl Aufsätze u Beobacht a d ges Med Ser 5 p 29 1783

7 Lacher Deutsch Arch f klin Med 27 268, 1880

8 Glassner Fortschr a d Geb d Röntgenstr 24 268, 1916, München med Wchnschr 63 19, 1916

9 Froriep Kleinert's Allg Repertorium der gesammten deutsch med-chir Journalistik, Leipzig, N S 1 53 (Oct-Dec) 1837

10 Meckel Cited by Zwanziger, Dissertatio inauguralis medico-chirurgica de hernia diaphragmatica Halae, 1819, p 26

great curve a little upward and downward, and is so exceedingly concave that the pylorus almost touches the cardia. The duodenum, by means of a great opening in the left side of the diaphragm, descends into the abdomen and here receives the bile-duct. Making a great curve downward, it returns into the thorax by the same aperture, and passes into the jejunum. This (the jejunum) with the ileum fills almost the whole cavity. The cecum is placed at the top of the left pleural sac. From this the ascending colon descends as far as the diaphragm, then making an obtuse angle, the transverse colon turns backward to the seventh dorsal vertebra. From here the ends of the transverse colon and the descending colon proceed outward (from the pleural cavity) and descend to the external margin of the opening, where they depart into the abdomen. Here everything is as usual. The colon, with looser folds than usual, is affixed to the walls of the thorax. The opening in the diaphragm measures 2 inches. Furthermore the very little developed left lung measures scarcely an inch in length, and equals scarcely a tenth part of the well developed right lung.

Further comment is unnecessary. Suffice it to say that both Fromeys and Meckel's cases were clear examples of hernia of the diaphragm.

Laennec¹¹ described a case of left-sided eventration in 1819, but his interest is confined to the auscultatory signs. Lawrence's case¹² (1852) was discovered at necropsy. The first case in which there is any note of clinical examination is that of Marsh¹³ (1867) who quotes Paget as follows:

There appeared, notwithstanding this defect (of the diaphragm, discovered postmortem), to be sufficient respiratory power. The patient breathed very forcibly in his dyspnea, and in auscultating him the movements of the chest appeared just as in an ordinary double pneumonia. The stomach was thought to be, as it was found, very high up, but nothing unusual attracted Mr. Archer's or my attention, the left side of the chest certainly moved freely, and the patient often lay on his right side.

That the side which was the seat of the eventration "certainly moved freely" was apparently contrary to the expectations of the examiners. The reports by Tennant¹⁴ (1894) and Thoma¹⁵ (1882) deal purely with the pathologic findings in genuine cases of eventration. Crispino's case, according to Sailer and Rhein,¹⁶ was doubtless one of eventration. The original article was not accessible.

The case of Struppler,¹⁷ described in 1901, is of some interest. Struppler himself, with the aid of the roentgen ray, made a diagnosis of hernia of the diaphragm. His arguments in favor of this diagnosis, however, are far from convincing, and his published skiagrams appear to show clearly an abnormally high, but intact, left diaphragm. One of them depicts a stomach tube, filled with mercury, lying in the

11 Laennec *L'Auscultation Mediate*, Paris 1 429, 1819

12 Lawrence *Lancet* 2 327, 1852

13 Marsh *Lancet* 1 298, 1867

14 Tennant *Edinburgh M J* 40 29 (July) 1894

15 Thoma *Virchow's Arch f path Anat* 88 515, 1882

16 Sailer and Rhein *Am J M Sc* 129 688, 1905, Crispino. Cited by Sailer and Rhein

17 Struppler *Deutsch Arch f klin Med* 70 1, 1901

stomach beneath the diaphragm. One is inclined to consider this case as an example of eventration, as have Arnsperger,¹⁸ Bayne-Jones,¹⁹ Glassner, and others. The case is the first to be studied with the roentgen ray, and it is immediately apparent that this procedure is so far from being a diagnostic aid in the differentiation of hernia from eventration that it precipitates a polemic which has raged unabated ever since.

From the article of Neisser²⁰ (1901) one might conclude that he had really observed cases of eventration of the diaphragm, associated, possibly, with aplasia of the lung, but his description suggests much and establishes little. The case described first by Widenmann²¹ in 1901, later also by Glaser²² and Fraenkel,²³ with pathologic findings by Benda,²⁴ was a classical one. Widenmann did not definitely decide the question between hernia and eventration, and Glaser and Fraenkel added nothing in their reports. Widenmann observed a difference in the excursion of the two sides of the thorax, there being a very slight impairment in the lower portion on the affected side. To Glaser the excursion appeared to be symmetrical. The fluoroscope merely added to the confusion. The necropsy findings were characteristic of eventration, the affected half of the diaphragm being the seat of a "lipomatous pseudo-hypertrophy" (Benda). Doering's case²⁵ (1902) seems well substantiated.

The celebrated case of Friedrich Schneider is of interest from several points of view. The first mention of him, according to Neumann,²⁶ occurred in 1890, when he was described by Stinzing as an example of congenital dextrocardia. From that time until his death in 1912 he went about presenting his diagnostic problem to one clinic after another and eventually became the center of a small polemic. He was roentgenographed so diligently that he acquired a burn, from which one infers, as Neumann suggests, that skiagraphy scarcely belongs in the category of diagnostic aids. The next published account of him is by Hirsch,²⁷ in 1900, who made a diagnosis of hernia of the diaphragm on the basis of roentgenographic findings. His skiagrams strongly suggest eventration, but Hirsch does not mention the latter

18 Arnsperger *Deutsch Arch f klin Med* **93** 88, 1908

19 Bayne-Jones *Arch Int Med* **17** 221 (Feb) 1916

20 Neisser *Ztschr f klin Med* **42** 88, 1901

21 Widenmann *Berl klin Wchnschr* **38** 279, 1901

22 Glaser *Deutsch Arch f klin Med* **78** 370, 1903

23 Fraenkel *Deutsch med Wchnschr, Vereins-Beilage*, No 48 p 343, 1902

24 Benda Cited by Glaser, loc cit

25 Doering *Deutsch Arch f klin Med* **72** 407, 1902

26 Neumann *Deutsch med Wchnschr* **45** 905, **45** 937, 1919, *Munchen med Wchnschr* **64** 719, 1917

27 Hirsch *Munchen med Wchnschr* **47** 996, 1900

even as a possibility Glassner states that Schneider's case was described by Volkmann, and that Becker, considering the case as an example of dextrocardia, made it the subject of his inaugural dissertation, Jena, in 1904. Schneider was carefully studied in 1905 by Hildebrand and Hess,²⁸ who were enabled to satisfy themselves of the correctness of their diagnosis of eventration by means of Schlippe's procedure of measuring the respiratory variations in intragastric pressure. The authors take issue with Hirsch over the interpretation of the skiagrams, maintaining that they portray clearly the presence of eventration. A year later, Lotze²⁹ referred to Schneider as a case of diaphragmatic hernia and was promptly rebuked by Hess.³⁰ The last chapter in this little drama was written in 1912 by Von Eggeling,³¹ whose report of the necropsy findings amply substantiated the diagnosis of eventration made by Hildebrand and Hess.

Sailer and Rhem (1905), in reporting a case of eventration, state that the affected half of the diaphragm was moderately thin, but microscopically was found to contain a fair amount of muscular tissue, "which accounted satisfactorily for the respiratory movement," the latter being noted as equal on the two sides. They conclude that, "in eventration of the diaphragm, the respiratory movements are normal." Reference to this will be made again. Wieting's case³² (1906) is notable for two reasons. It was the first example of right-sided eventration, and with it was associated a hernia of the diaphragm. Hamdi, who described this case previously, mentions nothing but the hernia. Wieting goes back over the gross pathology and demonstrates clearly that an eventration also existed, for the entire right phrenic leaf was found to be a "fascia-like structure without muscle fibers," whereas the left diaphragm was found to be normal. It is interesting to note that Glassner, in reporting his own case of right-sided eventration ignores Wieting.

Cases described in the period from 1907 to 1914 by Herz,³³ Arnspurger, Koniger,³⁴ Scholz,³⁵ Franck,³⁶ Appel,³⁷ Becker,³⁸ Scheideman-

28 Hildebrand and Hess. *Munchen med Wchnschr* **52** 745, 1905.

29 Lotze. *Deutsch med Wchnschr* **32** 1662, 1906.

30 Hess. *Deutsch med Wchnschr* **32** 1990, 1906.

31 Von Eggeling. *Munchen med Wchnschr* **59** 2284, 1912.

32 Wieting. *Deutsch Ztschr f Chir* **82** 315, 1906.

33 Herz. *Wien klin Wchnschr* **20** 1463, 1907.

34 Koniger. *Munchen med Wchnschr* **56** 282, 1909.

35 Scholz. *Berl klin Wchnschr* **48** 339, 1911.

36 Franck. *Beitr z klin Chir* **74** 358, 1911.

37 Appel. *Zur Kenntnis der Eventratio Diaphragmatica*, Inaugural Dissertation, Greifswald, 1911.

38 Becker. *Fortschr a d Geb d Rontgenstr* **17** 183, 1911.

del,³⁹ Malkow,⁴⁰ Bergmann,⁴¹ Kayser,⁴² Haase,⁴³ Reuss,⁴⁴ Baetge,⁴⁵ Krause,⁴⁶ and Manges and Wessler⁴⁷ may doubtless be classed with more or less accuracy in the category of eventration. With a few exceptions, the accounts are occupied chiefly with monotonous discussion of contradictory interpretations of roentgenograms. The cases of Beltz⁴⁸ (1910) and Motzfeldt⁴⁹ (1913) rest on necropsy findings. Fischer⁵⁰ (1914) makes mention of a case of eventration which "developed during convalescence from double pneumonia and typhoid fever." The diagnosis was made by a probatory thoracotomy. The inadequacy of his description leaves one in considerable doubt as to the correctness of the diagnosis of eventration.

Kienbock⁵¹ (1914) describes a case in which the diagnosis of eventration was confirmed at the time of an operation for relief of pyloric stenosis. This is probably the same Kienbock^{51a} who seven years previously described a case of diaphragmatic hernia. Apparently, the author never considered it in any other light, yet it has been cited by many as an instance of eventration. It is impossible to decide the question from a perusal of the original description, although the published roentgenogram, half schematic, suggests eventration. In the absence of supporting data, however, the diagnosis of eventration must be met with skepticism.

Glassner (1916) describes two cases as examples of eventration, in one of which the right diaphragm was the part affected. In this connection he ignores Wieting's case entirely, and rejects the right-sided case of Eppinger⁵² (1911) for the reason that it was merely mentioned, not described. Two of Eppinger's three cases were substantiated by necropsy, however, and it is not at all clear that any of them should be discarded. Glassner's case, then, properly becomes the third to be described as an instance of right-sided eventration of the diaphragm.

The five cases reported in 1916 by Weil⁵³ must be regarded with some skepticism in view of the inadequacy of the author's description.

39 Scheidemandel. *Munchen med Wchnschr* **59** 2168, 1912

40 Malkow. Cited by Bergmann (*loc cit*)

41 Bergmann. *Ergeb d inn Med u Kinderh* **12** 327, 1913

42 Kayser. *Fortschr a d Geb d Rontgenstr* **20** 240, 1913

43 Haase. *Wien klin Wchnschr* **26** 193, 1913

44 Reuss. *Deutsch med Wchnschr* **39** 743, 1913

45 Baetge. *Deutsch Arch f klin Med* **110** 49, 1913

46 Krause. *Deutsch Ztschr f Nervenhe* **47** 328, 1913

47 Manges and Wessler. *Med Rec* **86** 134, 1914

48 Beltz. *Munchen med Wchnschr* **57** 1006, 1910

49 Motzfeldt. *Deutsch med Wchnschr* **39** 312, 1913

50 Fischer. *Med Rec* **86** 653, 1914

51 Kienbock. *Fortschr a d Geb d Rontgenstr* **21** 322, 1914

51a Kienbock. *Ztschr f klin Med* **62** 321, 1907

52 Eppinger. *Allg u spez Path d Zwerchfells*, Suppl zu Nothnagel's spez Path u Therapie, Wien u Leipzig, 1911

53 Weil. *Vereinigung der Kriegsarztl beschaft Aerzte*, Strassburg, Jan 25, 1916, abstr in *Deutsch med Wchnschr* **42** 744, 1916

The cases had previously been "diagnosed" as (1) cardiac enlargement, the precordia extending $7\frac{1}{2}$ cm to the right of the midsternal line, (2) pulmonary affection, (3 and 4) pleurisy, depending on dulness and enfeebled breath sounds at the base of the left lung, (5) stomach and intestinal disease. All were diagnosed correctly as eventration, according to Weil, by the use of the roentgen ray.

The fourth case of eventration of the right side of the diaphragm was described in 1916 by Bayne-Jones. The diagnosis was made purely from physical examination, although the exact method by which the conclusion was reached does not appear from the author's description of the clinical findings. Roentgenograms and laparotomy confirmed the clinical impression. Wood⁵⁴ (1916) presents a case of eventration diagnosed from the roentgen ray picture. His inaccurate statements concerning the diaphragm and its participation in thoracic excursion betray his ignorance of the subject.

Betchov has written an interesting article (1917) in which he reviews some of the literature and reports a case. He admits frankly his confusion in attempting to establish a diagnosis, which was finally made by Professor Bard purely from the roentgen-ray findings. Betchov refers to a case described in 1917 by Weinberger⁵⁵ as an example of eventration. Weinberger's diagnosis, however, is hernia, and a perusal of his article does not lead to any other opinion. Minowski's case⁵⁶ (1917) is without doubt genuine. Neumann (1919) presents with necropsy findings a classical case of eventration of the diaphragm. His review of the literature is limited to German sources. He states that Lorey, in 1912, presented five cases,⁵⁷ two of which were substantiated by necropsy,⁵⁸ and that more recently Roesch⁵⁹ has described a case. Neither of these reports has been accessible. His discussion of the possible experimental production of eventration, although without the scope of this paper, is very interesting, and one feels inclined, without consulting the references he cites, to agree with him that the complete picture of eventration may well be produced in man by injury to the phrenic nerve.

Reports of cases of eventration by Andree⁶⁰ and Assmann,⁶¹ in 1918, appear plausible. The case of Aronson⁶² (1918) may possibly be considered as an example of right-sided eventration, although the

54 Wood Surg, Gynec & Obst **23** 344, 1916

55 Weinberger Munchen med Wchnschr **64** 624, 1917

56 Minowski Berl klin Wchnschr **54** 541, 1917

57 Lorey Cited by Neumann, Deutsch med Wchnschr **45** 905 1919

58 Lorey Cited by Neumann, Deutsch med Wchnschr **45** 905, 1919

59 Roesch Cited by Neumann

60 Andree Med Klin **14** 990, 1918

61 Assmann Fortschr a d Geb d Rontgenstr **26** 1, 1918

62 Aronson New York M J **108** 196, 1918, Med Rec **93** 37, 1918.

author's vague description of the findings raises much doubt. Laparotomy was undertaken for the purpose of resecting a congenital megacolon, but if the diagnosis was confirmed at this time, the report of it is extremely unconvincing. If genuine, it is the fifth case of right-sided eventration of the diaphragm. Schwenke⁶³ (1919) has reported two cases which he believes to be examples of eventration. His description is fairly convincing. Schlecht and Wels⁶⁴ (1920) have published a case of eventration, the diagnosis being confirmed by laparotomy. After the patient recovered from the operation, the authors injected air into his peritoneal cavity for purposes of roentgenographic study. The resulting pictures display the silhouette of the eventrated half of the diaphragm very effectively. They have also produced a pneumoperitoneum "without difficulty" in a patient with hernia of the diaphragm.

Funk and Manges⁶⁵ (1920) reported a case of eventration, the diagnosis being made by the roentgenogram. The patient was sent to the roentgen-ray laboratory with a diagnosis of hydropneumothorax. After seeing the picture, the authors re-examined the patient and discovered that they would have been able to arrive at the correct diagnosis from physical examination alone if they had been careful to observe the movement of the costal margins. Funk⁶⁶ states that there was "distinct widening of the subcostal angle on inspiration, a widening due principally to greater divergence of the left costal border." This is the only case in the literature in which the examiner made any observation of the excursion of the costal margins considered apart from the excursion of the arches of the ribs.

In concluding a critical analysis of the literature, one finds himself unable to make a definite statement as to the number of genuine cases of eventration which have been studied. The criteria by which the diagnosis has been determined are extremely vague and uncertain in a great many cases. A statistical venture on my part results as follows *

	Left	Right
Cases in which the diagnosis of eventration is regarded as proved	18	4
Cases in which the diagnosis of eventration is not proved, but is regarded as reasonably certain	41	2
Total	59	6 65

The first group includes, besides the cases substantiated by necropsy or laparotomy in which original articles have been consulted (1) two cases of Lorey cited by Neumann as having been controlled by

63 Schwenke *Deutsch med Wchnschr* **45** 1191, 1919

64 Schlecht and Wels *Fortschr a d Geb d Rontgenstr* **27** 244, 1920

65 Funk and Manges *Med Rec* **98** 289, 1920

66 Funk *M Clinics N America* **4** 1058 (Jan) 1921

*See summary of bibliography

necropsy, (2) the case of Funk and Manges, (3) my case. The reason for the inclusion of the last two is that in both cases the diagnosis was based on some knowledge of the physiology of the intercostal muscles and diaphragm. Statistics compiled by Franck, Motzfeldt, Bergmann, Eppinger, Bayne-Jones, and Neumann, I have shown to be inaccurate. The cases described by Hoffmann⁶⁷ as examples of so-called "rudimentary eventration," and "chronic habitual magenblasse" do not properly belong in the category of true eventration of the diaphragm. The case described by Otten and Schefold,⁶⁸ regarded as eventration by many writers, is also rejected for reasons which will appear later. Fischer's case, although supposed to be substantiated by thoracotomy, is classed as not definitely proved.

In searching for an example of association of eventration of the diaphragm with aplasia of the lung on the affected side, one finds a statement by Glassner to the effect that the coexistence of the two conditions has never been established. Its occurrence is faintly suggested by Neisser and Haase purely on roentgenographic grounds. In the cases coming to necropsy, the state of the lung on the affected side is mentioned only superficially. Motzfeldt makes the statement that in his case the left lung was small and exhibited an anomalous lower lobe—scarcely to be interpreted as aplasia. In this connection, articles by Abercrombie,⁶⁹ Clark,⁷⁰ Kronig,⁷¹ Fitzgerald and Everett,⁷² Ponfick,⁷³ and Barlow⁷⁴ may be consulted, but none of these contains any note of the condition of the musculature of the diaphragm, and none can be considered as an example of eventration. Similarly, the case of Schmit, cited by Baetge and Glassner, cannot be regarded as an instance of true eventration. My case is, I believe, the first in which aplasia of the lung has been shown to accompany the condition of eventration of the diaphragm.

3 THE DIAGNOSIS

Krause said, "Diseases of the diaphragm are proportionately very seldom diagnosed. This is due both to the slight understanding of the physiology of the diaphragm, and the scarcity of the pathological condition." The critic perceives that the second sentence constitutes within itself a fallacy of induction which becomes still greater if he appreciates, not the "slight understanding," but the total want of

67 Hoffmann *Munchen med Wchnschr* **54** 112, 1907

68 Otten and Schefold *Deutsch Arch f klin Med* **99** 468 1910

69 Abercrombie Cited by Clark, *Am J M Sc, N S* **20** 106, 1850

70 Clark *Am J M Sc, N S* **20** 106, 1850

71 Kronig *Centralbl f inn Med* **19** 178, 1898

72 Fitzgerald and Everett *Brit M J* **2** 664, 1900

73 Ponfick *Virchow's Arch f path Anat* **50** 633, 1870

74 Barlow *Brit M J* **1** 14, 1880

knowledge of the physiology of the diaphragm betrayed by most writers on the subject. In particular, there is no evidence on record that the diagnosis of eventration of the diaphragm has ever been made by clinical application of such knowledge. Instead, various conflicting criteria and mechanical devices have been introduced as alleged aids in diagnosis. Some of these may be detailed.

1 Schlippe's procedure of measuring the variations in intragastric pressure during different phases of respiration



Fig 1—Roentgenogram of Case 1 Viewed from the front

2 Jamin's procedure of electrical excitation of the phrenic nerve

3 By means of the fluoroscope (a) The observation of so-called "paradoxical movement" of the affected half of the diaphragm (b) Observation of the respiratory movement of the mediastinum

4 By means of the fluoroscope and roentgenogram, the identification of the linear, arched shadow, which extends from the mediastinum to the lateral thoracic wall

5 The employment of pneumoperitoneum

From a priori considerations, one might expect to gain pertinent information by the use of the device proposed by Schlippe. It was mainly through its employment that Hildebrand and Hess were enabled to arrive at a correct diagnosis in the case of the long-suffering Schneider. They demonstrated an inspiratory rise and expiratory fall in intragastric pressure, which is the reverse of what should occur if the stomach had invaded the thorax through a hole in the diaphragm. In view of their success one is surprised to find that subsequent investigators largely failed to utilize the method, but this fact is doubtless significant of its impracticability. Beigmann says that it may be of use, but fails to employ it in his own case. Minkowski found, with costal respiration, an inspiratory fall of intragastric pressure, and with abdominal breathing, an inspiratory rise. The inference to be drawn from this is obvious. A little consideration will suggest many more situations in which the results of the procedure must prove equivocal, and one need not go farther to show that it is worthless as a help in diagnosis.

Jamin's proposal to distinguish between normal and atrophic diaphragmatic musculature, and hence between hernia and eventration of the diaphragm, by fluoroscopic observation of the extent of the excursion of the two halves of the diaphragm caused by electrical excitation of the phrenic nerves in the neck, has not proved of service. Such a procedure, if employed in a normal subject in the interim between respirations, should theoretically eliminate the effect on the excursion of the diaphragm produced by the conflicting factor of activation of the intercostal muscles, and, therefore, should result in a degree of excursion directly proportional to the strength of the stimulus, the amount of diaphragmatic musculature, and the amount of normal conducting tissue in the phrenic nerve. Practically, however, the method is a very crude one, for the stimulation of the intact phrenic nerve in the human subject cannot be performed without collateral excitation of the scalene muscles, and possibly also of the upper intercostals, so that one cannot be sure that he is obtaining the necessary isolated effect predicated above. Furthermore, the cases under consideration are not in all respects normal subjects. If a diaphragm which is composed of normal muscle fibers, but is the seat of a hernia, shall have acquired a high fixation to the thoracic wall through the formation of pleural synechia (personally observed at operation), the test must necessarily fail. Again, the test will be positive for eventration in a patient who has normal musculature in the diaphragm, but has suffered an impairment in the conducting property of his phrenic nerve. Further factors complicating the results of this procedure are readily called to mind, and, as is to be expected, the test has not fared well in the hands of many investigators. Scheidemandel

observed total absence of excursion of the diaphragm on the affected side in his case of eventration. Reuss noted only a slight difference in the amount of excursion on the two sides. Minkowski states ambiguously that stimulation of the phrenic nerve gave contraction of the diaphragm on the affected side. Andree found slight downward excursion of the diaphragm on the affected side. Neumann noted only a minimal response in a case of gunshot injury of the phrenic nerve.



Fig 2—Roentgenogram of Case 2 Viewed from the back

Assmann concludes, from the experience of others, and on theoretic grounds, that the method is not of any service in determining the diagnosis.

Hildebrand and Hess were the first to observe under the fluoroscope the so-called “paradoxical movement” (inspiratory ascent, expiratory descent) of the affected half of the diaphragm. Their case was later proved by necropsy to be one of eventration. Lotze, because of the

absence of this paradoxical excursion, made the diagnosis of eventration in his case, but the necropsy by Risel⁷⁵ revealed, not eventration, but hernia of the diaphragm. Schlecht and Wels observed the absence of paradoxical movement in their case, which was later proved by operation to be one of eventration. Neumann likewise noted its absence in a proved case of eventration. It was present in Bayne-Jones' proved case. Minkowski observed its presence during costal breathing, and absence with abdominal respiration. Assmann comments on the unreliability of the sign. These are the most conspicuous examples, although many other writers have remarked its presence or absence. In view of these contradictory observations, it is scarcely desirable to undertake a critique of the theory of "paradoxical" movement. As a test, it has no diagnostic significance.

Observation under the fluoroscope of respiratory excursion of the mediastinum has been made three times (Herz, Otten and Schefold, Minkowski). Herz noted in his case an inspiratory excursion of the mediastinum away from the affected side toward the sound side. He quotes Holzknacht, who first described this phenomenon in connection with unilateral paralysis of the phrenic nerve, as follows:

Since in the quiet expiratory phase an equal pressure exists in both halves of the chest and falls only in the healthy side during inspiration, and since the intrabronchial communication cannot bring about an equilibrium quickly enough both pressures are first of all equalized through displacement of the mediastinum which is effected by the stronger pull of the healthy side.

The explanation is essentially correct. The inspiratory increase of negative intrapleural pressure is normally brought about by an increase in the anteroposterior and transverse diameters of the thorax due to the activation and excursion of the intercostal muscles, with a concomitant maintenance, or increase, of the longitudinal diameter due to the activation and excursion of the diaphragm. In normal inspiration, the negative intrapleural pressure rises in value at an equal rate on the two sides, with consequent immobility of the mediastinum. If one-half of the diaphragm be deprived of its activation, the longitudinal diameter of the chest on that side will not be maintained or increased, but will be diminished, the negative intrapleural pressure will increase at an unequal rate on the two sides, and the mediastinum will consequently undergo inspiratory excursion toward the side which develops the greater negative pressure. In eventration of the diaphragm, or unilateral paralysis of the phrenic nerve, therefore, the mediastinum will exhibit inspiratory excursion toward the healthy side.

The observation of Otten and Schefold here comes into view. In a patient supposed to have eventration of the left half of the diaphragm,

⁷⁵ Risel. *Munchen med Wchnschr* 54 637, 1907

the heart and mediastinum were seen under the fluoroscope to move toward the left side during inspiration, with return toward the right side during expiration. This means that there must have been a greater negative intrapleural pressure created on the left side, but it is impossible to conceive of this if the left half of the diaphragm were the seat of aplasia. In the absence of other factors which might explain this phenomenon, we are, therefore, forced to reject this case from the category of eventration.

In discussing respiratory excursion of the mediastinum there is one other factor which must be considered. With normally functioning intercostal muscles and diaphragm, negative intrapleural pressure rises in value at an equal rate on the two sides only if the extensibility of the two lungs be unimpaired and there is no bronchial obstruction to the ingress of air. The following experiment illustrates the point.

EXPERIMENT—A medium-sized dog (under morphin-chloretone anesthesia), with intact scaleni, intercostal muscles and diaphragm, breathing in a perfectly normal fashion, was given a complete obstruction to all except the lowest main bronchus on the right side, with consequent collapse of all except the lower lobe. When observed with the fluoroscope, the mediastinum and heart were seen to undergo an inspiratory excursion toward the right equal in extent to that exhibited in a downward direction by the right half of the diaphragm. Removal of the bronchial obstruction abolished all movement of the mediastinum, restoration of the obstruction caused the inspiratory excursion to reappear.

Negative intrapleural pressure increased at an equal rate on the two sides in this experiment. On the left side, it was equalized as it developed by the ingress into the bronchi of air under atmospheric pressure, on the right side, however, equalization of the rapidly rising negative pressure could not occur because of the bronchial obstruction, hence the disparity in pressure on the two sides, with resultant excursion of the mediastinum.

The third mention of mediastinal excursion is by Minkowski, who noted the movement toward the healthy side during inspiration. Mediastinal excursion in man is seldom a conspicuous phenomenon, even when conditions for its production are ideal—a fact which probably explains a want of any note of it in so many of the case reports of eventration of the diaphragm. The dog's mediastinum is less rigid, and therefore more easily responds to inequalities in negative intrapleural pressure.

A great part of the literature on eventration and hernia of the diaphragm is occupied with a discussion of the identity of the arched line which is seen in the skiagram to reach from the mediastinum to the lateral thoracic wall—the "bogenförmige Schattenlinie" of the Germans. Is it the silhouette of the strongly up-bellied, thinned-out

diaphragm, or is it the shadow cast by the distended magenblasse which has invaded the thorax through an aperture in the diaphragm? The arguments advanced pro and con, in the same case, and in different cases, remind one of the old shadow-picture of the horse and rider. At one instant he seems to be riding toward the observer, and in the next he is riding away. It has been sufficiently demonstrated that the assumption simply of a point of view toward this bow-shaped, linear silhouette cannot by any flight of the imagination be construed as diagnostic proof.

Recently Schlecht and Wels made roentgenograms of a case of eventration of the diaphragm after the production of a pneumoperitoneum. The procedure apparently offers a sure means of identifying the arched line mentioned above. In the presence of a hernia of the diaphragm, however, the dangers attendant on possible entrance of air into the pleural cavity must certainly be considered.

The key to the differentiation between hernia and eventration of the diaphragm lies in an application to the problem of a knowledge of the physiology of the diaphragm and intercostal muscles, a correct conception of which was denied to the earlier writers. Recently, however, this subject has been abundantly expounded by Hoover.⁷⁶ Given a case for differentiation, how shall we determine whether we have to deal with a weakened diaphragm standing in a very high position without solution of its continuity, or a diaphragm standing in a normal position, but the seat of an opening through which some of the abdominal viscera have invaded the thorax? We shall attempt to answer the question.

The movement of the costal margins during inspiration is the resultant of the activation of the intercostal muscles and the activation of the diaphragm. In this contest, the diaphragm labors under a distinct mechanical disadvantage, due to the considerable disparity which exists between the resultant line of traction of the diaphragm and the anatomic curve of the diaphragm. Consequently, under normal conditions the intercostal muscles obtain the mastery over the diaphragm with the result that the costal margins throughout their whole extent move away from the median line during inspiration. If the curve of one-half of the diaphragm becomes accentuated without the formation at the same time of synechia between the thoracic wall and the phrenic leaf, the result is an increase in the disparity between the resultant line of traction of the diaphragm and the curve of the diaphragm.

⁷⁶ Hoover, C. F. The Functions of the Diaphragm and Their Diagnostic Significance, *Arch Int Med* **12** 214 (Aug.) 1913. Diagnostic Signs from the Scaleni, Intercostal Muscles and the Diaphragm in Lung Ventilation, *Ibid* **20** 701 (Nov.) 1917. The Functions of the Intercostal Muscles, *J. A. M. A.* **73** 17 (July 5) 1919. The Diagnostic Significance of Inspiratory Movements of the Costal Margins, *Am J M Sc* **159** 633 (May) 1920.

This half of the diaphragm is thus reduced to a still greater mechanical disadvantage in its contest with the intercostal muscles for the mastery of the costal margin, and the latter, as a consequence, exhibits inspiratory divergence from the median line considerably in excess of the normal. In this exaggerated outward excursion the entire costal margin, from the xiphoid to the posterior axillary line, participates, and in comparison with the contralateral margin, its movement is not only one of greater extent, but it is accomplished more actively. In my case this asymmetry of movement of the costal margins was very striking.

In detecting movement of the costal margins, it is very important that the examiner combine inspection and palpation, and in palpation it is essential that the fingers be placed, not carelessly over the costal arches, but on the extreme ends of the ribs. It must be understood that the excursion of the costal borders (extreme ends of the ribs) is entirely independent of the excursion of the arcs of the ribs. The rib arc always moves upward and outward in "bucket-handle" fashion in response to normal activation of the intercostal muscles, whereas the direction of movement of the costal margins is the resultant of activation of the intercostals and diaphragm. One may find inspiratory convergence of the costal borders, but normal "bucket-handle" excursion of the arc of the rib, and conversely, impaired excursion of the rib arc but exaggerated inspiratory divergence of the costal margin. The last was a conspicuous feature in my case, and is of especial interest in a critical analysis of the observations on respiration of the thorax recorded in the literature on eventration of the diaphragm. Among the reports of cases which are adjudged genuine, one finds that twenty-one writers have made some mention of thoracic excursion. Eight have noted that the excursion on the two sides was equal, ten, that there was "slight lagging" of the affected side, one, that there was no movement whatever, and one, that the movement was better on the affected side. The subject is usually disposed of in a few vague words, and the observation is without significance either to the writer or to the reader. Reference may here be made to the obscure and inaccurate statements of Sailer and Rhem, Eppinger, and Wood. Observation of the movement of the costal borders has never before been made except by Funk, whose statement I have previously quoted. With this exception, all have failed to perceive the fact that the excursion of the arcs of the ribs and that of the costal margins (ends of the ribs) are quite independent of each other, and, although the former may conceivably be unaffected, or slightly impaired, depending on the degree of impairment of the extensibility of the lower lobe of the lung, caused by some additional factor, such as chronic pleurisy, the latter may at the same time exhibit an exag-

gerated outward excursion for an entirely different reason, viz, the great disparity which exists, in eventration, between the resultant line of traction and the curve of the diaphragm

In hernia of the diaphragm, however, with which eventration has been invariably confounded, the conditions are entirely different. Here one finds a diaphragm, not aplastic and in a high position, but with intact muscle fibers and occupying an approximately normal position. It is, of course, conceivable that a very extensive aperture, occupying one-half or two-thirds of the phrenic leaf, would diminish the muscular power of the diaphragm to such an extent as to allow exaggerated outward excursion of the costal margins from intercostal activation, regardless of the anatomic curve of the diaphragm. This is the exceptional case, however, and one in which the diagnosis is not ordinarily a problem. In hernia of the diaphragm, then, the factors which control the movement of the costal margins are not essentially changed, and as a consequence one finds their excursion normal in extent and bilaterally symmetrical. If there should be any asymmetry of movement, it occurs as lessened outward movement on the affected side, due either to flattening of the diaphragm from the downward traction exerted on it by the abdominal viscera which have become adherent to its structure at the hernial orifice, or to the acquisition of a high insertion of the diaphragm from the formation of pleural synechia between it and the thoracic wall.

REPORT OF CASES

CASE 1—J. A. R., male, aged 20 years

Diagnosis—Aplasia of the right lung and right half of the diaphragm, associated with cor dexter

Present Illness—Patient is not ill except for a slight furunculosis of the right axilla. He comes into the hospital for purposes of observation.

Past History—Patient states that he has never been ill, but was always vigorous and able from childhood to compete fairly well with his playmates. He can engage in moderate exercise and perform a moderate amount of physical work without discomfort of any kind. Violent exercise, however, causes him to experience intense throbbing of his heart and a sense of weakness in the arms and legs, but he does not become short of breath. Patient discovered when still a child that his heart lay on his right side. If asked by his teacher to place his hand over his heart he would put his left hand across his right chest. When he was 8 years of age he was informed by medical examiners that his heart lay on the right side.

Physical Examination—Thorax. There is a marked scoliosis of the mid and lower thoracic vertebrae, the concavity being to the right. The lower end of the sternum is well to the left, a perpendicular dropped from the middle of the thyroid cartilage intersecting the right sternal line at the level of the fifth rib. The transverse and anteroposterior diameters of the right thorax are considerably less than those of the left. Hepatic dulness is encountered at the level of the seventh rib in the midaxillary line on the right, whereas on the left the lower border of the pleural sinus extends to the ninth rib in the midaxillary line. On the right there is a marked diminution in the undulatory movement of the upper five ribs, together with distinct impairment in excursions.

sion of the arches of the lower seven ribs. In an area on the right side bounded above by the clavicle, below by the liver, medially by the sternum, and laterally by the midaxillary line there is dulness to percussion, and a total want of tactile fremitus and breath sounds, in other words, there is no evidence that this area is occupied by lung. There is evidence, however, that the remainder of the right thorax, viz, the supraclavicular, suprascapular, interscapular, infrascapular, and the axillary and infra-axillary regions as far anteriorly as the midaxillary line, are occupied by normal lung. In these areas the percussion note is of the normal pitch, duration and resistance, the breath sounds are of normal character and intensity, and the tactile fremitus is normally transmitted. The left lung is normal throughout.

Heart. On the right side of the chest anteriorly there is plainly visible a diffuse precordial impulse occupying a quadrilateral area bounded above by the second rib, below by the fifth rib, medially by the right border of the sternum, and laterally by the anterior axillary line. In addition, there is a sharply localized systolic impulse, resembling a "choc en dome," visible at the right second intercostal space in a line intersecting the junction of the inner and middle thirds of the clavicle. Over this area one palpates a strong systolic impulse and diastolic impact. The character of the heart sounds is consistent with the location of the apex at the fifth rib in the right parasternal line, and the conus arteriosus of the right ventricle at the second right intercostal space in the line above mentioned.

Costal Margins. With the patient in the recumbent position, the entire right costal margin, from the xiphoid to the posterior axillary line, is seen to undergo an inspiratory divergence from the median line distinctly in excess of that of the left—evidence of a want of activation of the right half of the diaphragm in its effect on the costal margins.

Clinical Diagnosis.—Taking into account the history, which clearly proved that the patient had never been ill, and the physical findings, which proved that the patient could not have a disease of his lung or pleura, Dr Hoover made a diagnosis of aplasia of the right lung and right half of the diaphragm, associated with an embryonic displacement of the heart upward and to the right caused by this failure of development of the right lung and diaphragm.

Fluoroscopic Examination.—Under the fluoroscope, a great disparity between the longitudinal diameters of the two sides of the chest was apparent from the high position of the dome of the liver and the low position of the vault of the left diaphragm. During inspiration the right half of the diaphragm underwent no excursion of its own, but was drawn passively downward and to the left by the very lively excursion of the left diaphragm. There was no "paradoxical" movement of the right diaphragm, and the mediastinum could not be seen to undergo any excursion. The entire right half of the thorax was less transparent than the left. A striking feature was the fact that the heart failed almost entirely to cast a shadow. Its borders were extremely faint, and would probably not have been visible at all except for their slight mobility. This phenomenon was interpreted as being due to the fact that there was a want of aerated lung surrounding the heart. The roentgenograms were of the same character (Fig 1). Whether the aorta arched over the right or the left bronchus was not discernible.

It should also be mentioned that the patient had a hypospadias and that both his testes were intra-abdominal. His vital capacity was 3,200 cc. A boy of his height (5 feet 8 inches) should have at least 4,500 cc.

CASE 2—E. C. W., male, aged 29 years.

Diagnosis.—Hernia of the left diaphragm.

Present Illness.—Patient states that on arising in the morning he is seized with a severe pain in the epigastrium, which gradually passes away after he has maintained the erect posture for a period of from two to three hours. The pain is usually associated with vomiting, especially if he eats breakfast. By

noon the discomfort has entirely disappeared, and he is always able to enjoy a hearty lunch and to retain it without difficulty. This disappearance of symptoms with the assumption and continued maintenance of the erect position has been constant throughout. There is nothing in the history to indicate disease of the intrathoracic organs, or local disease of the stomach or other abdominal viscera.

Past History—When the patient was about 9 years old he was run over by a 1500 pound delivery wagon, one of the rear wheels passing over his abdomen at about the level of the umbilicus. Two ribs on the left side were fractured. Following this injury he was in bed for six weeks, having pain in the abdomen and left shoulder. From that time, the patient was always more or less disturbed by the epigastric pain described, although it would occasionally be absent for long periods. During the past few years, however, the trouble has been growing distinctly worse. (Note: The full history of this injury was not obtained until after the diagnosis had been made. Patient at first dated the onset of the trouble only a few years previously.)

Physical Examination—*Thorax* Symmetrical, with no disproportion in the relations of the antero-posterior, transverse, or longitudinal diameters. The volume, density, and extensibility of the right lung are normal. At the base of the left thorax posteriorly, in an area bounded above by the sixth rib, medially by the spine, and laterally by the posterior axillary line, the percussion note is dull and has a tympanitic quality, tactile fremitus is increased, and the breath sounds are high pitched and tubular in character. No râles. The tympanitic quality of the percussion note is interpreted as Skodaic resonance due to relaxed lung. In this area the coin sound and succussion splash are occasionally demonstrable. The percussion note, tactile fremitus, and breath sounds are normal in character throughout the remainder of the left lung. The excursion of the arcs of the ribs, and that of the costal margins, is equal and active on both sides. The fact that the costal borders undergo symmetrical inspiratory divergence is proof of normal activation of both halves of the diaphragm, for normally activated intercostal muscles, unopposed by the factor of activation of the diaphragm, cannot fail to bring about exaggerated inspiratory divergence of the costal margins. The left half of the diaphragm, therefore, cannot be the seat of aplasia. Furthermore, a normal excursion of the arches of the ribs cannot take place in the presence of impaired extensibility of the underlying lung. Hence, chronic pleurisy does not explain the dullness at the base of the left thorax, nor does partial infiltration of the lung account for the tympanitic percussion note. Hydropneumothorax is similarly excluded. The physical signs could be interpreted only as dependent on the invasion of the thorax by some abdominal viscus.

Heart There is no visible or palpable precordial activity. Percussion shows that the heart occupies a normal position, the right border being located at the right sternal line, and the left at the left midclavicular line. Auscultation reveals nothing abnormal during systole or diastole.

Clinical Diagnosis—From the physical signs, without having obtained the history of injury, Dr Hoover made the diagnosis of hernia of the left diaphragm.

Fluoroscopic Examination—Under the fluoroscope there was no disparity to be seen between the longitudinal diameters of the two sides of the chest, and the diaphragm underwent a normal excursion on both sides. When the patient was given barium mixture to drink, the stomach was seen to lie almost entirely in the left pleural cavity, its upper level reaching to the third rib anteriorly. There was no displacement of the heart (Fig 2).

COMMENT

In a great proportion of the cases of hernia and eventration of the diaphragm, as reported in the literature, there has been a striking uniformity in the physical signs from palpation, percussion, and auscul-

tation It is for this reason that a study of the evidences of activation of the diaphragm is of paramount importance This uniformity did not extend in its entirety to our two cases, partly because of the co-existing aplasia of the lung in the case of eventration In spite of this fact, the most important difference in the physical signs of the two cases was apparent in the behavior of the costal margins, the interpretation of which enabled us to make the diagnosis by direct physical examination without instrumental aid

CONCLUSION

1 The literature on the subject of "eventration" of the diaphragm is critically reviewed, chiefly from the standpoint of diagnosis

2 Diagnostic criteria are critically discussed Emphasis is laid on the necessity for a careful study of the inspiratory excursion of the costal margins in the attempt to differentiate eventration from hernia of the diaphragm

3 The evidences for activation, or want of activation, of the diaphragm in hernia and eventration are illustrated by the presentation of cases

SUMMARY OF BIBLIOGRAPHY

Articles dealing with cases in which the diagnosis of "Eventration of the Diaphragm" is regarded as proved Ref Nos 1, 12, 13, 14, 15, 16, 19, 21, 22, 23, 26, 28, 32, 48, 49, 51, 52, 58, 64, 65, 66

Articles dealing with cases in which the diagnosis of "Eventration of the Diaphragm" is not proved, but is regarded as reasonably certain Ref Nos 3, 6, 8, 11, 17, 18, 20, 25, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 50, 53, 54, 56, 60, 61, 62, 63

Articles dealing with cases in which the diagnosis of "Eventration of the Diaphragm" is neither proved nor reasonably certain Ref Nos 5, 9, 10, 51a, 55, 67, 68

Articles inaccessible to the author supposed to present cases of Eventration Ref Nos 57, 59, and

Cordier *Progres med* **35** 193 (May 1) 1920

Kwasek Cited by Schlecht and Wels, loc cit

Articles dealing with diagnostic signs from the intercostal muscles and the diaphragm are listed under Ref 76

Further references, not cited in text

Editorial *Med Rec* **93** 72, 1918

Giffin *Ann Surg* **55** 388, 1912

Grosser *Wien klin Wchnschr* **12** 655, 1899

Kienbock *Wien klin Wchnschr* **11** 538, 1898

Leichtenstern *Berl klin Wchnschr* **11** 497, 515, 539, 1874

Lenormant *Presse med* **20** 350, 1912

Stockton *Buffalo M J N S* **38** 97, 1898

THE BIGEMINAL PULSE IN ATRIOVENTRICULAR RHYTHM ³

PAUL D WHITE, M D
BOSTON

Bigeminal pulsation in atrioventricular rhythm is extremely rare. In 1915, the electrocardiograms of an unique case seen at the Massachusetts General Hospital were published ¹. During the past year, a second case showing the same condition has been examined, also at the Massachusetts General Hospital. No other cases have been reported in the literature, so far as I am aware ².

Atrioventricular rhythm, once called "nodal rhythm," is that cardiac rhythm arising from the atrioventricular node (of Tawara) which lies in the connective tissue below the endocardium of the right auricle just above the septal edge of the tricuspid valve ring. Impulses arising in this node travel in both directions, upward to produce an upside-down contraction of the auricles, and downward to produce ventricular systole. If the *a-v* node stimulates the ventricles alone while the sino-auricular node (the normal "pacemaker" of the heart) stimulates the auricles, auriculo-ventricular dissociation occurs either of the complete heart block type or of ventricular escape type ¹ but not atrioventricular rhythm. The position of the unusual pacemaker in the atrioventricular node or bundle, and the degree of resistance to the spread of the excitatory wave determine whether ventricles or auricles beat first. In both my cases the *a-v* impulse reached the ventricles definitely before reaching the auricles.

Atrioventricular rhythm is of little significance clinically. It usually means depression of the sino-auricular node and thus escape of the lower node, probably largely due to vagal overactivity. It may, however, mean excessive irritability of the *a-v* node, giving rise to paroxysmal tachycardia of this origin. Both my cases resulted apparently from sino-auricular depression, and in the second case ventricular

³ From the Cardiac Clinic of the Massachusetts General Hospital.

¹ White, P. D. Arch Int Med **16** 517 (Nov.) 1915, Arch Int Med **18** 244 (Aug.) 1916.

² Since this paper was written, examples of the phenomena of the bigeminal pulse in atrioventricular rhythm have been published by Gallavardin ³ and by Bishop ⁴. Bishop makes no particular note of the bigeminy, but Gallavardin and Graves explain their case as I have done, they produced the bigeminy (one example only in their paper) by vagal pressure in *a-v* rhythm. The vagal pressure prolonged the *R-P* interval to such an extent that the ventricle responded to the late *P*.

³ Gallavardin, L., and Graves, L. Bradycardie Nodale Permanente. Etude du Rythme Atrioventriculaire, Arch du mal du coeur, **14** 11, 1921, [Tracing VII].

⁴ Bishop, L. F. Specific Action of Atropin in Relieving Certain Irregularities of the Heart Beat, J A M A **77** 31, (July 2) 1921, [Fig 3].

escape appeared in some records and atrioventricular rhythm in others, showing the close relationship between these two phenomena

The bigeminy consists of the sandwiching of one retrograde auricular contraction between two practically normal ventricular contractions. The mechanism was discussed in my former paper¹ and probably is as follows. Ordinarily in atrioventricular rhythm the auricular excitation occurs just before, with, or immediately after the ventricular excitation wave. Rarely, the auricular impulse is so much delayed that the auricular excitation wave induces in its turn a second ventricular contraction giving rise to the bigeminal pulse of atrioventricular rhythm. Thus there is a sandwiching of an auricular contraction between two ventricular beats (Fig 4). A diagram illustrating this mechanism will be found in the paper describing the first case¹. The only other reasonable explanation of the bigeminy is that abnormal auricular beats are excited mechanically by the idioventricular contractions and that these in turn are followed by ventricular responses. In support of this theory in the present case is the fact that at first (Feb 15, 1920) the auricular complexes were upright even when the bigeminy occurred, and that later (March 10), when they were inverted, they did not always occur at the same time interval after the ventricular complexes in the spontaneous rhythm. The chief reason for considering them of lower nodal origin is the fact, that in this case, as in the first case reported, nervous influences markedly affected the *R-P* interval. For example, in Figure 6 exercise shortened the *R-P* interval (from 0.38-0.53 sec to 0.19 sec), probably by sympathetic nerve action, to such a degree that the bigeminy was interrupted. As the atrioventricular rate decreased after the exercise, the *R-P* interval increased (in one-half minute to 0.3 sec) and the bigeminy reappeared (Fig 6b). Thus, a paradoxical slowing of the pulse occurred with exercise, the rate dropping to 40 immediately after exercise, while half a minute later it rose to 60 because of the bigeminy, although the actual rate of stimulus production in the atrioventricular node had dropped to 30 (Fig 6b). Also, atropin sulphate not only quickened the atrioventricular nodal rate from 26 to 41, but it shortened the *R-P* interval from 0.55 sec to 0.28 sec (Fig 7). This shortening, however, was not sufficient to abolish the bigeminy.

The *P-R* interval in the periods of bigeminal pulse was always shorter, often very much, than the *R-P* interval (0.18 sec as compared with 0.4 sec [Fig 4]), except after atropin when they were about equal to 0.28 sec, and also after exercise (Figs 6 and 7). Forward conduction across the auriculonodal junction is more rapid than retrograde conduction ordinarily, and so would explain this difference when the heart was uninfluenced by atropin and exercise. The probable explanation of lengthening of the *P-R* interval after atropin and exercise, an unexpected finding usually, lies in the fact that the auricular impulse

coming earlier than usual finds the conduction system less completely recovered and so more resistant to the passage of an impulse. As has been noted after exercise, if the retrograde auricular impulse follows the ventricular complex by a still shorter interval there is no second ventricular complex at all.

REPORT OF CASE

A M, male, carpenter, 48 years old, born and raised in New England, was examined Feb 25, 1920

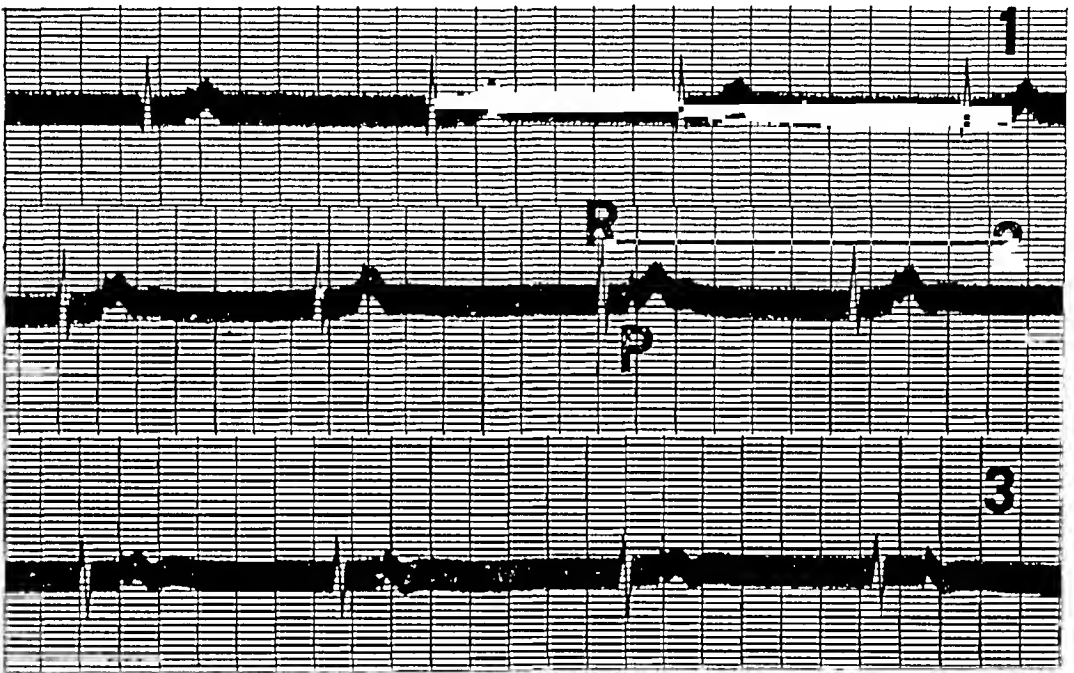


Fig 1—Electrocardiogram of A M, taken Feb 25, 1920, showing Leads I, II and III in sequence. Sino-auricular bradycardia and ventricular escape occur with variable *R-P* intervals. Rate 46. In this, as in the following electrocardiograms, scale on abscissa = 0.2 second, on ordinate = 10^{-4} volt.

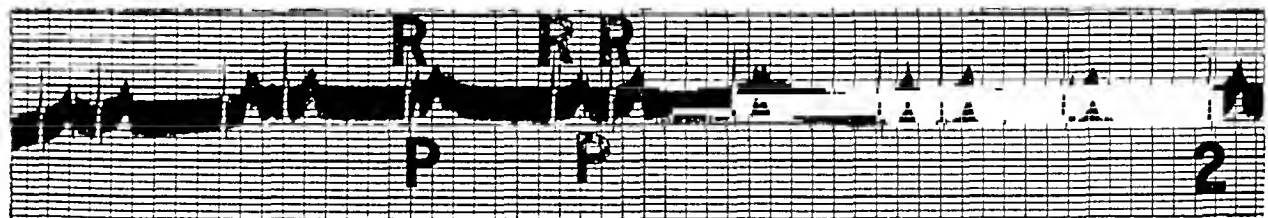


Fig 2—Electrocardiogram, Lead II, of A M, Feb 25, 1920, showing occasional bigeminal pulse complicating sino-auricular bradycardia and ventricular escape.

Past and Family History—These are of no interest, except for diphtheria at the age of 7 years, influenza in 1918, and considerable financial worry for the past two years. Two years ago he was examined and passed by two insurance companies. Has used no tobacco or alcohol and little coffee.

Present Illness—This started about one year ago when he began to tire easily and to suffer from aching in his legs. He noticed that at times of dizziness and weakness his pulse went down to 15, but he never fainted. Epigastric discomfort often has bothered him, but he has had no severe pain or jaundice.

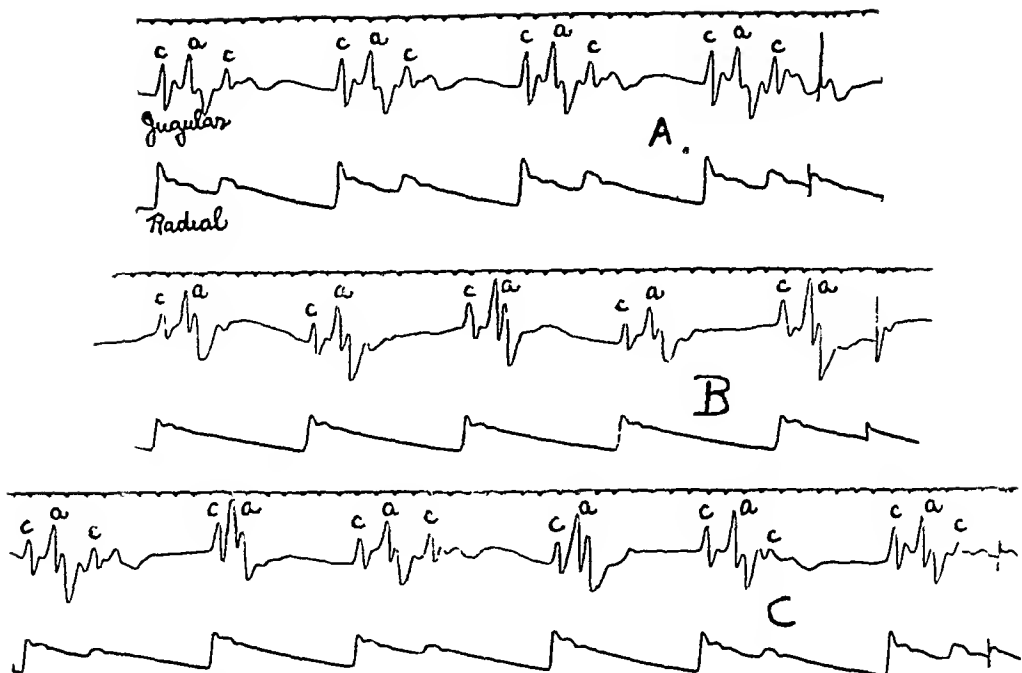


Fig 3—Polygrams of radial and jugular pulse of A M, Feb 25, 1920, showing (A) bigeminal pulse with c-a-c waves in jugular tracing, (B) regular pulse with c-a waves in jugular tracing, (C) alternate bigeminal and single pulsations, the former occurring with the longer c-a intervals

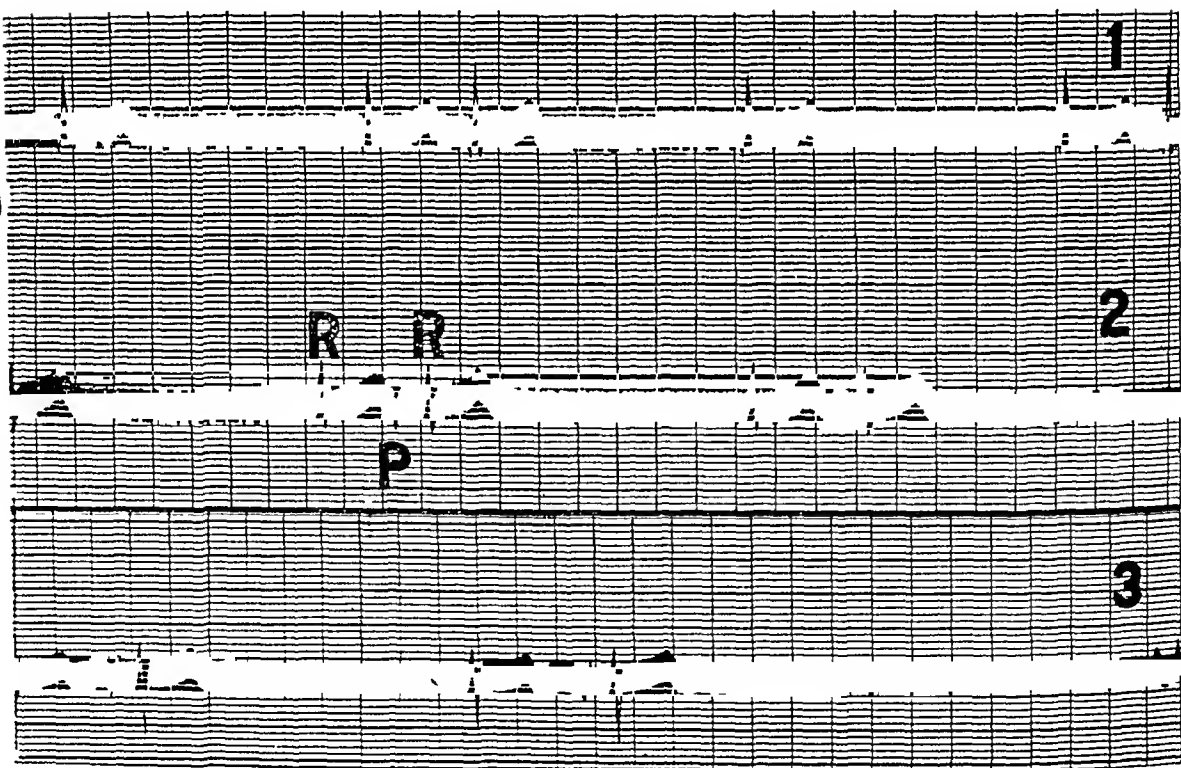


Fig 4—Leads I, II and III of A M, March 10, 1920, showing atrioventricular rhythm with very slow and varying rate of a-v nodal pacemaker, in Lead I, 38, Lead II, 27 and in Lead III, 25 and less In Lead III, the rate of stimulus production is so reduced that the depressed sino-aortic node finally escapes in its turn after a three second interval



Fig 5—Lead II of A M March 10, 1921, showing sino-auricular nodal escape after marked slowing of the atrioventricular nodal pacemaker with resumption of *a-v* rhythm. Spontaneous variation in rate

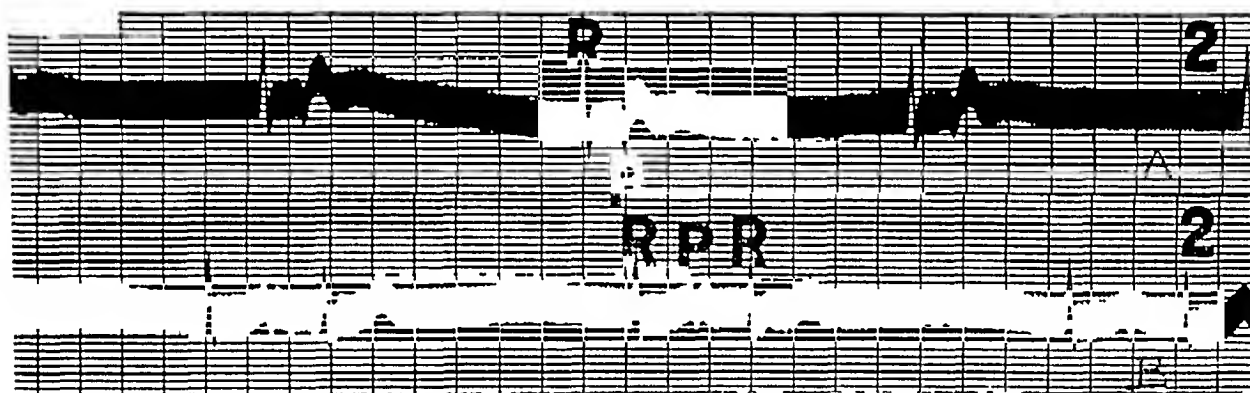


Fig 6—Lead II, March 10, showing the effect of exercise (A) taken one-half minute after exercise. The R-P interval is here so reduced that the bigeminy has disappeared (B) Taken one minute after exercise, with lengthening of the R-P interval and reappearance of the bigeminy

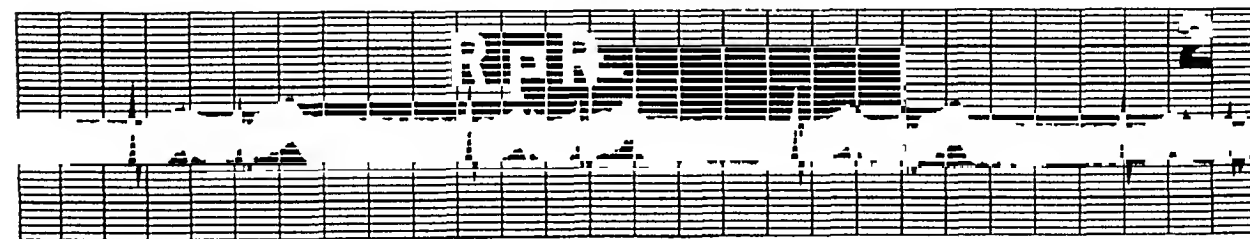


Fig 7—Lead II, March 10, showing the effect of atropin sulphate, $\frac{1}{30}$ grain, injected subcutaneously. The rate of stimulus production in the atrioventricular node is increased, and the R-P interval is decreased but not sufficiently to abolish the bigeminy. The P-R interval (of the latter half of the couple) is increased beyond the pre-atropin measurement

There has been no relation of discomfort to food. Physical examination by his physician, Dec 6, 1919, was negative, except for a pulse rate of 48 and blood pressure of 160 mm Hg systolic and 100 mm diastolic. For more than a month his pulse rate ranged from 56 to 32. There was no relief from small doses of digitalis. He kept at work. In February his pulse rate usually was 36, but varied from 28 to 38, in March it varied from 34 to 58. March 17, 18, 19, 20, 21 and 22, his morning and evening pulse rates were 34, 36, 34, 34, 44, 44 and 36, 38, 36, 54, 52, 58, respectively.

Physical Examination—Feb 25, 1920. Patient was a slender, fairly developed middle-aged man without dyspnea, cyanosis or edema. His heart was slightly enlarged to the left, with apex impulse in the fifth space, no increased supracardiac dullness, short systolic murmur at apex, pulse rate, 45, blood pressure 160 mm mercury systolic, and 85 mm diastolic. Reflexes normal.

Wassermann reaction negative

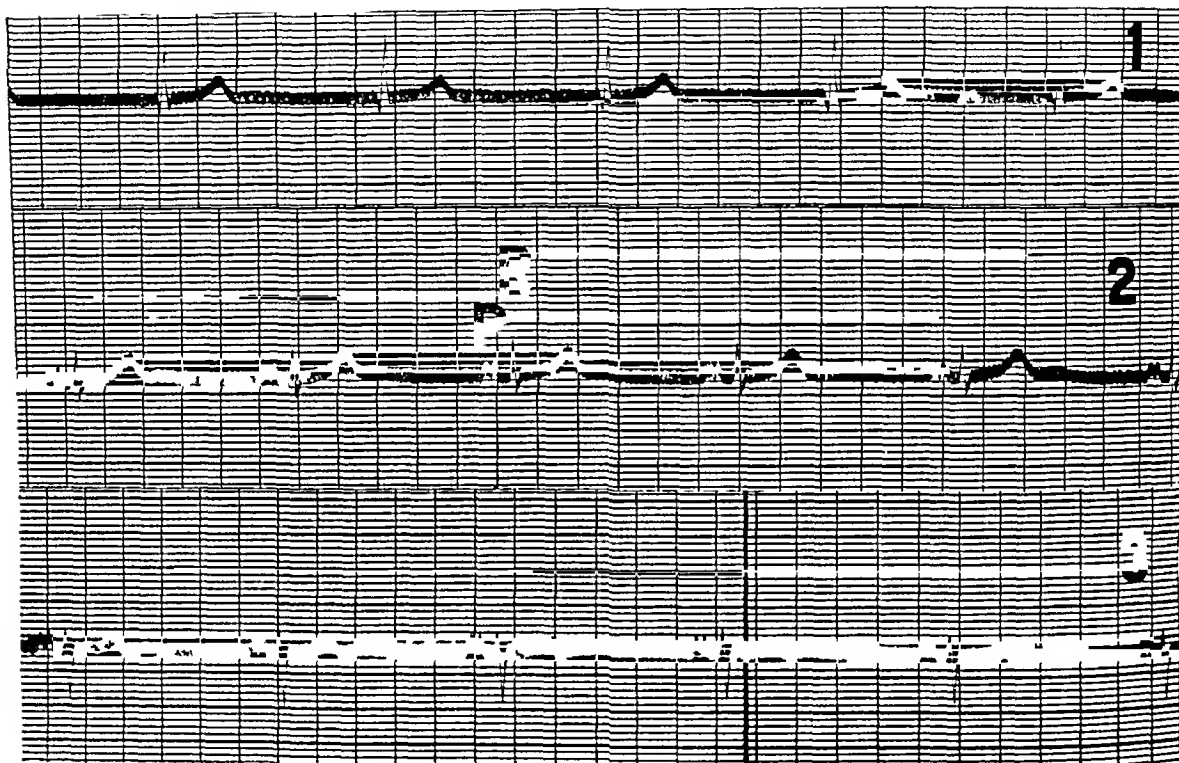


Fig 8—Electrocardiogram, Leads I, II and III of A. M., March 31, 1920, showing normal rhythm, bifurcated *P* wave in Leads II and III and normal *P-R* interval. Rate 55.

Electrocardiographic and Polygraphic Studies—Electrocardiogram, Feb 25, 1920, showed sino-auricular bradycardia and ventricular escape and occasionally bigeminy when the auricular excitation wave stimulated the ventricle when not refractory (Figs 1 and 2). A polygram of radial and jugular pulse tracings taken February 25 shows at times a bigeminal pulse (Fig 3). The ventricular wave *c* always precedes the auricular wave *a* whether there is a bigeminal pulse or not. The earlier the auricular impulse occurs the less chance there is of a ventricular response. As seen in the figure the *c-a* interval is slightly longer in the bigeminal pulse tracing than in the other. The difference is very slight, we are close to the borderline, in fact so close that at times in the same strip of tracing both single and bigeminal pulsation occurred (Fig 3c). Similar polygrams were obtained during the periods of atrioventricular rhythm.

March 10, 1920, the electrocardiogram (Fig 4) showed atrioventricular rhythm with inverted *P* wave falling very late after the *R* and followed by a second *R* wave (bigeminy). On two occasions (Figs 4 and 5) the atrioventricular node ceased function for three seconds, the sino-auricular node thereupon resuming its activity and giving rise in its turn to a ventricular response.

Effect of exercise on the bigeminal pulse of atrioventricular rhythm. A rapid walk of 400 yards increased the rate of the atrioventricular pacemaker (Fig 6) from 25-35 to 40 but decreased the actual pulse rate from 55 to 40 because of the disappearance of the bigeminy. With the quickening of the *a-v* rhythm there was a decrease in the *R-P* interval, as mentioned above. It was this very decrease that undoubtedly did away with the bigeminy for the *a-v* bundle was probably still refractory when the auricular impulse returned to it, one-half minute later (Fig 6) the *R-P* interval had lengthened out again (from 0.2 to 0.3 second). This allowed the bigeminy to reappear and the pulse rate rose to 60 although the actual *a-v* rate had dropped to 30.

Atropin sulphate, $\frac{1}{30}$ grain, was injected subcutaneously, also March 10, 1920, and thirty minutes later the *a-v* rate had risen from 30 to 40 and the pulse rate from 60 to 80, the bigeminy persisting (Fig 7). Here, in spite of the increase in *a-v* nodal rate, the *R-P* interval did not decrease sufficiently to cause the bigeminy to disappear as exercise had done. The *R-P* interval did, however, decrease to 0.25 second.

Both after exercise and after atropin the second ventricular complex of the bigeminal pulse in the electrocardiogram was not quite normal in appearance. There was slight aberration, consisting chiefly of a widening of the *s* wave. This is also suggested in similar complexes before exercise and atropin were tried.

March 25, 1920, a roentgen-ray study of a barium meal showed no abnormality of the gastro-intestinal tract.

March 31, 1920, an electrocardiogram (Fig 8) showed perfectly normal rhythm with a *P-R* interval of about 0.16 second and with a split *P*. The patient now felt well and seemed again normal.

Three months later (June, 1920) the patient was apparently normal.

SUMMARY

The case of a patient is recorded who from some unknown cause of probable vagus overactivity developed sino-auricular bradycardia (or *s-a* block) with ventricular escape, the rhythm changing later to atrioventricular in type and finally returning to normal—all in the course of a few weeks. Little, if any, clinical evidence of disease existed at any time. When the atrioventricular rhythm was slowest and the backward conduction of the impulse from the *a-v* node to the auricle the most retarded, a curious bigeminy occurred with the sandwiching of an auricular complex between two ventriculars. This is the second observation of this phenomenon recorded.

THE SYNTHESIS AND ELIMINATION OF HIPPURIC ACID IN NEPHRITIS A NEW RENAL FUNCTION TEST *

PRELIMINARY PAPER

F B KINGSBURY, PH D AND W W SWANSON, B S
MINNEAPOLIS

Since the time when Bunge and Schmiedeberg¹ showed that the perfused dog kidney could effect the synthesis of hippuric acid from benzoic acid and glycine, it has been assumed by investigators, from time to time, that the synthetic ability of the kidney for the formation of hippuric acid could be used as an index of renal function Rowntree and Geraghty,² in their paper on the use of phenolsulphonephthalein as a means of testing renal function, enumerate among other renal tests that of the synthesis of hippuric acid More recently, Violle³ reported that in nephritis the formation of hippuric acid is much less than in normal individuals after giving 0.5 gm doses of sodium benzoate and collecting the twenty-four hour specimens He found that in some cases the amount of extra hippuric acid corresponding to the amount of ingested benzoate was not excreted under forty-eight hours The work of Kingsbury and Bell⁴ has thrown doubt on the view that the kidney is the only place in which hippuric acid is synthesized in the dog, and Friedmann and Tachau⁵ have proved that in the rabbit the perfused surviving liver can effect the synthesis of hippuric acid Lackner, Levinson and Morse⁶ studied the effect of poisoning dogs with hydrazine sulphate on the synthesis of hippuric acid, and found

* From the Biochemical Laboratory of the Department of Physiology, University of Minnesota

* A preliminary report was made in Chicago, December, 1920, before the Federation of American Societies for Experimental Biology

1 Bunge, G, and Schmiedeberg, O Ueber die Bildung der Hippursaeure, Arch exper Path u Pharmacol **6** 233, 1876

2 Rowntree, L G, and Geraghty, T J An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, J Pharmacol & Exper Therap **1** 579, 1910

3 Violle, P L, Ann de med **7** 272, 1920, Abst J A M A **75** 435, 1920, Lancet **1** 884, 1920

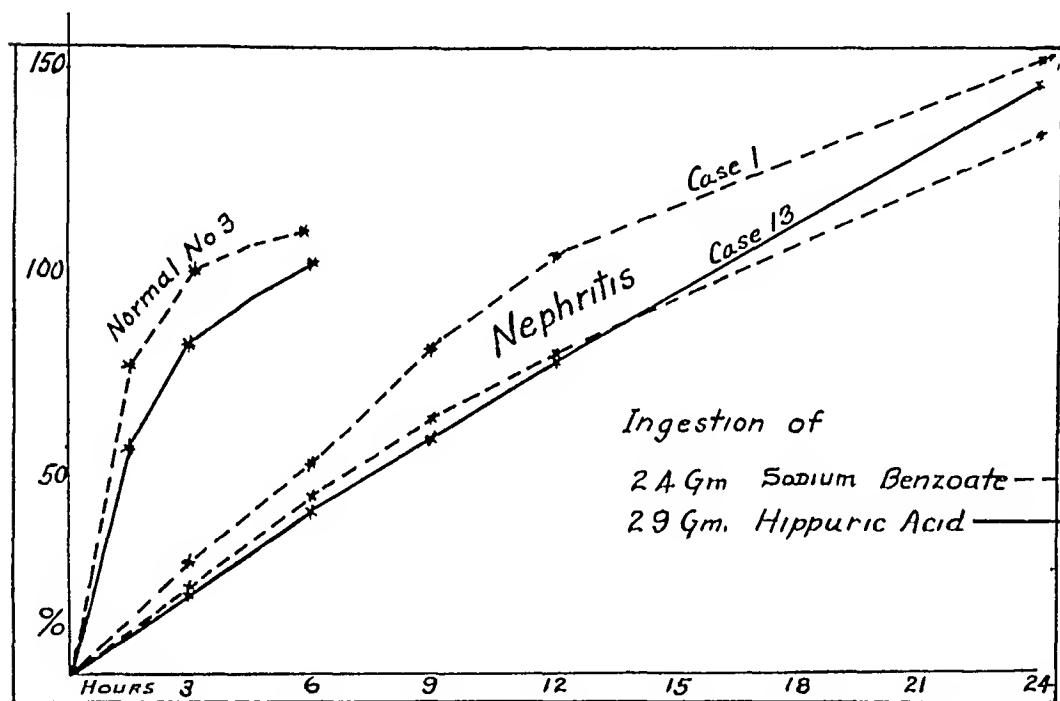
4 Kingsbury, F B, and Bell, E T The Synthesis of Hippuric Acid in Nephrectomized Dogs, J Biol Chem **21** 297 (June) 1915

5 Friedmann, E, and Tachau, H Ueber die Bildung des Glykokolls im Tierkoerper, 1 Mitteilung, Synthese der Hippursaeure in der Kanninchenleber, Biochem Ztschr **35** 88, 1911

6 Lackner, E, Levinson, A, and Morse, W The Rôle of the Liver in Hippuric Acid Synthesis, Biochem J **12** 184, 1918

that with the liver extensively damaged, as it is by this poison, the synthesis of hippuric acid was greatly diminished. No damage to the kidney after use of hydrazine sulphate was demonstrated.

Jaarsveld and Stokvis,⁷ using a slightly modified Bunge and Schmiedeberg method of analysis for the determination of benzoic and hippuric acids, estimated the amounts of these substances in the twenty-four hour urine of hospital patients suffering from various forms and degrees of nephritis after giving doses of sodium benzoate ranging from 0.3 to 4.5 gm. As controls, normal individuals were given amounts of sodium benzoate ranging from 0.4 to 2.0 gm. In four experiments with the controls these authors found no free, or unconjugated, benzoic acid excreted, but in the pathologic cases the percen-



tage free of the total benzoic acid excreted varied from zero per cent in the contracted kidney type of nephritis to as high as 100 per cent in the chronic and acute parenchymatous type, in other words, no synthesis at all. They concluded that an extensive damage to the kidney parenchyma resulted in a lessened ability of this organ to function in synthesizing hippuric acid. They also found that the hippuric acid excretion was apparently related to the excretion of albumin, for in those urines containing much albumin smaller quantities of hippuric acid were always found than in urines free from albumin.

7 Jaarsveld, G. J., and Stokvis, B. J. Ueber den Einfluss von Nierenerkrankungen auf die Bildung von Hippursäure, *Arch. exper. Path. u. Pharmacol.* 10: 268, 1879.

Weyl and Anrep⁸ showed that in cases of pulmonary tuberculosis, ileotyphus and myelitis decubitus the normal twenty-four hour excretion of hippuric acid varied from 0.011 to 0.083 gm, and that unconjugated benzoic acid was always present. In the only case in which this was estimated it amounted to 0.23 gm as compared with 0.06 gm hippuric acid found in the same urine. These authors used Bunge and Schmiedeberg's procedure.

Kronecker⁹ gave nephritics 0.5 gm doses of sodium benzoate and found that in one case of contracted kidney, 74 per cent of the total benzoic acid excreted in twenty-four hours was in the form of free benzoic acid and 26 per cent was combined. In a second trial with the same patient a few days later, under exactly similar conditions of experiment, he found that the relations between free and combined benzoic acids were practically the reverse from those of the first experiment.

It will be seen from these brief summaries of previous work on the synthesis of hippuric acid in nephritis and other pathologic conditions that no agreement exists and this is easily explained as being due entirely to the analytical methods used. These were in every case either Bunge and Schmiedeberg's or some slight modification of it. In any case, the specimens of urine, if not already alkaline, were made so with sodium carbonate, and evaporated to dryness prior to the extraction and weighing of the hippuric and benzoic acids. It has been shown, and repeatedly referred to by various investigators, that hippuric acid is hydrolyzed by alkali. The large amounts of free benzoic acid were found in the urines which we can suppose were the ones most alkaline or which had been subjected to longer treatment with alkali, for the results which are to follow show that in nephritis hippuric acid is synthesized completely and, as far as can be learned, at practically the same rate as in normal individuals. Free benzoic acid, at least only in traces sufficient to require from 0.1 to 0.2 cc of tenth normal sodium ethylate for every 100 cc urine used, is all that can ever be obtained from pathologic or normal urine, provided that steps are taken to avoid any accidental hydrolysis of the hippuric acid present.

In the present paper we have based our test for renal function not on the supposed function of the kidney to form hippuric acid, but on its ability to excrete it. We have studied the rate of hippuric acid elimination after equivalent doses of benzoic or hippuric acids over short as well as over long periods with normal and pathologic individuals by methods of analysis not open to the criticism referred to above.

⁸ Weyl, Th., and Anrep, B. Ueber die Ausscheidung der Hippursaeure und Benzoesaeure waehrend des Fiebers, *Ztschr. Physiol. Chem.* **4** 169, 1880.

⁹ Kronecker, F. Ueber die Hippursaeurebildung beim Menschen in Krankheiten, *Arch. exper. Path. u. Pharmacol.* **16** 344, 1883.

ANALYTICAL METHODS

Hippuric acid in the urines of our controls was analyzed by the Folin-Flanders' ¹⁰ method directly, with one modification, which will be mentioned in its place. It was necessary to devise a way to remove albumin from urine before this method of analysis could be used in the cases of those urines containing it, since it has been known for a very considerable number of years, that by proper oxidation of protein benzoic acid can be obtained. Kingsbury and Bell ¹¹ showed that egg albumin, when subjected to the Folin-Flanders procedure, yielded titratable acid, some of which was benzoic acid. We have confirmed this finding and give below the number of cubic centimeters of one-tenth normal ethylate equivalent to various amounts of egg albumin. This substance dissolved in 100 c c water was put through this method

0.50 gm egg albumin	3.4 c c
1.0 gm egg albumin	7.55 c c
2.0 gm egg albumin	16.8 c c
5.0 gm egg albumin	34.9 c c

REMOVAL OF PROTEIN FROM PATHOLOGIC URINE

One hundred c c albuminous urine in a casserole are treated with 4 drops of 0.1 per cent alcoholic solution of methyl red. This gives an acid reaction since our pathologic specimens are always collected in sufficient 2 per cent nitric acid more than to neutralize the alkali present in such specimens (15 c c have been found adequate for a three hour specimen). Raiziss and Dubin ¹² have shown that there is no appreciable hydrolysis of hippuric acid in rabbit urine collected in an excess of 2 per cent nitric acid. Sufficient normal sodium hydroxide is added, a drop at a time, to bring the color to the first definite yellow. The urine is now boiled to coagulate the protein, and during the boiling hydrochloric acid, approximately one-tenth normal, is added until a definite red coloration is noticed. Good coagulations are always obtained. This is the case whether the urine is at the first definite yellow of methyl red or at the first definite red, i.e. over a Ph range of from 5 to 6, approximately. The clear filtrate is filtered off, the precipitate of protein washed with two 100 c c portions of boiling distilled water, and the combined filtrates and wash waters evaporated to dryness after adding 10 c c of 5 per cent sodium hydroxide solution as in the regular procedure. The remainder of the method is exactly

¹⁰ Folin, O., and Flanders, F. F. A New Method for the Determination of Hippuric Acid in Urine, *J Biol Chem* **11** 257 (April) 1912

¹¹ Kingsbury, F. B., and Bell, E. T. The Synthesis of Hippuric Acid in Experimental Nephritis in the Rabbit, *J Biol Chem* **20** 73 (Jan.) 1915

¹² Raiziss, G. W., and Dubin, H. On the Estimation of Benzoic Acid in the Urine *J Biol Chem* **20** 125 (Feb.) 1915

that of Folin and Flanders, except the final stage referred to below. We have found that more consistent results, with better end points in the final titration of the resulting benzoic acid by the Folin-Flanders method, are obtained if the extract, instead of being drawn directly into a flask and titrated, is filtered through a dry filter paper, the 25 c c of neutral chloroform which is used to rinse the filter paper through which the extract has been passed also being used to rinse the separatory funnel from which the extract was drawn. The rinse chloroform is filtered through a second dry filter paper. This procedure removes all visible water. The only criticism which we have to make of the Folin-Flanders method is the length of time necessary for a determination, an alkali treatment of from two and one-half to four hours, an acid treatment of four and one-half hours and an extraction of from one-half to three-quarters of an hour longer. It seemed probable to us that a method could be devised which would take much less time without sacrificing any of the accuracy which is characterized by the Folin-Flanders method. We have done this, but we have not as yet given our method a sufficiently long trial to publish it. This we hope to do in the near future in *The Journal of Biological Chemistry*.

The method used for the determination of uncombined or free benzoic acid is that described by Raiziss and Dubin¹². Dakin,¹³ Lewis,¹⁴ Raiziss and Dubin¹⁵ and others have shown that in man benzoic acid is entirely converted into hippuric acid and excreted in this form. In pathologic cases our only information, as previously referred to, is a disagreement as to the excretion of benzoic acid or its salts. We have made the free benzoic acid determination in our pathologic cases a sufficient number of times, but have never found a titration figure in terms of c c of one-tenth normal sodium ethylate larger than that found for the same amount of normal urine, namely a blank of from 0.10 to 0.20 c c for 100 c c urine. Hippuric acid is the only form in which the total benzoic acid is excreted, in types of disease at least with which we have worked. As in the hippuric acid determination, protein if present in the urine, must be removed before the acidified urine can be extracted with toluol in making the determination for free benzoic acid. Our method for this is as follows:

A 100 c c sample of albuminous urine in a 200 c c volumetric flask containing four drops of 0.10 per cent methyl red solution is brought

13 Dakin, H. D. The Fate of Sodium Benzoate in the Human Organism, *J Biol Chem* **7** 103 (Jan.) 1910.

14 Lewis, H. B. The Synthesis and Rate of Elimination of Hippuric Acid After Benzoate Ingestion in Man, *J Biol Chem* **18** 225 (July) 1914.

15 Raiziss, G. W., and Dubin, H. The Synthesis of Hippuric Acid in the Animal Organism and the Occurrence of Free Benzoic Acid in the Urine, *J Biol Chem* **21** 331 (July) 1915.

to the first definite yellow by the addition of normal sodium hydroxid, then back to the first definite red by approximately one-tenth normal hydrochloric acid. Then, 0.5 gm of tannic acid in substance is added. After complete solution of this substance and the consequent precipitation of the protein, the liquid is made up to volume, filtered through a dry filter paper and a 100 c c aliquot analyzed according to the method of Raiziss and Dubin.¹²

The simple coagulation method for the removal of protein prior to the application of the Folin-Flanders method gave consistent results in our hands. These are shown in Table 1. The samples of urine, 100 c c each, contained from 0.5 to 1.0 gm egg albumin (the dry product) and the values obtained were compared in each case with those of a similar quantity of the same sample of urine not containing egg albumin.

The results shown in Table 1 prove that albumin can be removed from urine according to the method outlined above without altering

TABLE 1—DATA ON PRELIMINARY REMOVAL OF PROTEIN

Urine 1		Urine 2		Urine 3	
No Albumin Present, N/20 Sodium Ethylate Required, C c	1.0 Gm Albumin Present, N/20 Sodium Ethylate Required, C c	No Albumin Present, N/20 Sodium Ethylate Required, C c	1.0 Gm Albumin Present, N/20 Sodium Ethylate Required, C c	No Albumin Present, N/20 Sodium Ethylate Required, C c	0.5 Gm Albumin Present, N/20 Sodium Ethylate Required, C c
9.15	9.26	10.87	10.48	8.79	8.82
Average of 2 determinations	Average of 8 determinations	Average of 4 determinations	Average of 2 determinations	Average of 4 determinations	Average of 4 determinations

appreciably the true hippuric acid titration value. In practice, albumin was removed when the sample of urine gave a coagulable quantity, determined on a 5 c c test sample, using the same technic as that described above.

EXPERIMENTAL

Our basic dose of sodium benzoate or hippurate is the equivalent of 2.0 gm benzoic acid. The few exceptions to this are noted in the proper place. Since Lewis¹⁴ had shown that the excretion of hippuric acid following the ingestion of from 6 to 10 gm doses of sodium benzoate was very rapid, from 85 to 90 per cent of the dose being eliminated in from five to six hours, it seemed to us that with a dose of 2 gm the elimination would be more rapid, and this has been found to be the case. We have found that the elimination of hippuric acid after the ingestion of 2.4 gm sodium benzoate is 96 per cent of the benzoate ingested for a 2.4 to 3.25 hour period. Directly determined and indirectly estimated, from the curves of excretion, the average for three hours for nine normal individuals is 97 per cent.

In all cases, the sodium benzoate was dissolved in from 75 to 100 c c of water. This dose was taken and followed by half a glass of water used to rinse the container and glass from which the solution was taken. The benzoate was usually given between meals, either morning or afternoon, but in some cases one hour before breakfast, or with the meals. No difference could be noted either in the normal or pathologic cases due to the time of giving the sodium benzoate, with the exception of Case 12 Table 4, but we prefer the mid-morning or afternoon for this purpose. In every case care was taken to have the subject void urine immediately before taking the sodium benzoate. The diet was controlled only in so far as fruit in any form or cranberries were concerned. These were not allowed on the experiment day. The amount of water taken during the experiment period was not controlled in the normal cases, nor could any differences in the results, possibly due to this, be noticed. The water intake was controlled in the pathologic cases.

In those experiments in which hippuric acid was given this was dissolved at room temperature by the aid of sodium bicarbonate, avoiding an excess of the latter. The hippuric acid used in these experiments was for the most part synthesized from benzoyl chlorid and glycine in slightly alkaline solution, entirely freed from benzoic acid and recrystallized two or three times from hot water and the crystals thoroughly dried. Some of it was purchased from the Eastman Kodak Company¹⁶

Previous mention has been made of the fact that our pathologic specimens of urine were collected in 15 c c of 20 per cent nitric acid for each three hour specimen to prevent hydrolysis of the hippuric acid, and it should be noted that when the specimen of urine had to stand over more than six or eight hours before the analysis of it could be undertaken, it was preserved by adding 0.5 c c of a 10 per cent solution of thymol in chloroform. Such preservation makes no difference in the analytical results, as we have demonstrated several times.

The administration of salicylates, at least in any quantity, just before or during the experiment period should be avoided. Salicylates present in urines analyzed by the Folin-Flanders method yield results that are too high, due, probably, to the formation of picric acid or analogous substances which yield a certain titration value of their own. We have found that picric acid can be proved to be present when 10 gm salicylic acid or acetylsalicylic acid is put through the Folin-Flanders procedure. None of our normal or pathologic results were influenced in this way.

16 For this and certain other chemicals for research purposes acknowledgment is made to the Graduate School of the University of Minnesota

In general, we have found it advantageous to make the three hour pathologic specimens up to 500 cc and take 100 cc aliquots for analysis

The average daily excretion of hippuric acid is given in text books on physiological chemistry as from 0.7 to 1.0 gm, but the more accurate figures of Folin and Flanders indicate that it varies from 0.8 to 1.9 gm a day. This has been determined in the case of a normal man weighing approximately 90 kg and the results are shown in Table 2.¹⁷

The results shown in Table 2 confirm the findings of others that the hippuric acid output is fairly constant for the individual on a constant diet, and that fruit and vegetables increase it. The average daily excretion in this case on the mixed diet is 1.70 gm as hippuric acid, or a three-hourly excretion of 0.144 gm calculated as benzoic acid. On

TABLE 2—NORMAL HIPPURIC ACID EXCRETION

Date June, 1919	24 Hour Hippuric Acid, Gm	Diet
10	1.58	Mixed, including a fair amount of fruit
11	1.85	
12	1.51	
13	1.61	
14	1.80	
16	1.85	
17	1.60	Milk, cheese, cream and rock candy, but no fruit or vegetables
18	1.14	
19	0.85	
20	0.99	
21	0.87	
22	0.85	
23	0.90	
24	0.93	

the low hippuric acid forming, but high protein diet (from 20 to 22 gm total nitrogen in twenty-four hours) the output of hippuric acid, averaging the figures from June 19 to 24, inclusive, is 0.90 gm as hippuric acid, or a three-hourly excretion of 0.077 gm calculated as benzoic acid.

We have disregarded the ordinary three hour excretion of hippuric acid in the normal and pathologic cases, and have simply determined the amount of hippuric acid excreted in this time after the ingestion of the equivalent of 2 gm benzoic acid, expressing this in per cent of the ingested substance. Our figures, then, include this ordinary output which is too small relatively to affect the results of our comparison of the rates of hippuric acid elimination in normal and pathologic subjects under these conditions. Directly determined, the twenty-four hour output of hippuric acid on a fruit free diet was in Case 19, 0.25 gm,

¹⁷ Done by Mr. Wallace Cole in an elective course under the direction of F. B. Kingsbury.

in Case 20, 1.08 gm, and in Case 21, 0.321 gm, all calculated as benzoic acid. The three-hourly output in each case calculated as benzoic acid was 0.03, 0.14 and 0.04 gram, respectively.

Violle³ recently brought forth a renal function test based on the supposed ability of the kidney to synthesize hippuric acid. He has found that a forty-eight hour period is sometimes necessary in cases of nephritis for the synthesis of hippuric acid equivalent to 0.5 gm benzoic acid. Our findings, which were about half complete when the abstracts of Violle's paper were available, do not confirm those of Violle. The original article of Violle has not yet arrived, and we are, therefore, in ignorance as to more of the details of his work and particularly as to the analytical methods employed.

Case histories have been omitted from our paper, but all the clinical findings that could throw light on the results have been included. Results with the normal subjects are shown in Table 3, with pathologic subjects in Table 4.

DISCUSSION OF RESULTS

In summarizing our results, we have classified our patients into (1) Nephritics, (2) cardiac decompensation with passive congestion of the kidneys, and (3) miscellaneous cases. In Table 5, dealing with well marked cases of nephritis, it is seen that a low phenolsulphonephthalein test is associated with a low benzoate test. With the exception of Cases 1 and 8, with extremely low phenolsulphonephthalein tests these values ranged from 10 to 20 per cent for adults and were associated with benzoate tests of from 10 to 50 per cent. With twelve cardiac cases shown in Table 6, the phenolsulphonephthalein percentages varied from 20 to 47, the latter being the lower limit of what is regarded by some internists as normality, and are generally higher than with cases of nephritis. It is pointed out in this connection that the benzoate tests on these patients are generally relatively higher than the corresponding phenolsulphonephthalein tests, for instance, half of them by the former test ranging from 60 to 80 per cent, and half from 20 to 60 per cent, but none lower than 20 per cent. Generally, then, the benzoate test shows greater differences between these two types of cases than does the phenolsulphonephthalein test. In Table 4 it will be noted that in Cases 8 and 10 several benzoate tests were made, and that the values obtained fluctuated markedly. Apparently, this is due to the condition of the heart and its ability to maintain an adequate renal circulation during the time of the test. Fluctuations in the benzoate test when applied to the nephritic are by no means so marked. Case 13 showed steady clinical improvement from November 27 to December 8. During this period, the benzoate test percentages rose from 10 to 22 and the phenolsulphonephthalein values from 20 to 35 per cent in two hours.

TABLE 3—NORMAL INDIVIDUALS

No	Date 1920	Substance Ingested, Gm	Hippuric Acid Excreted			Sex
			Total, Calculated as Benzoic Acid, Gm	Total Time Elapsed After Ingestion of Substance, Hours	Total Excretion Per Cent of Substance Ingested	
1	June 23	2 4 A	1 12	1 25	56	M
			1 97	2 50	96	
			2 08	3 75	104	
	June 25	2 9 B	1 038	1 25	52	
			1 455	2 73	73	
			1 629	3 75	82	
	Sept 3	2 9 B	1 719	5 00	86	
			0 852	1 52	42	
			1 505	2 50	75	
			1 697	3 75	85	
2	June 1	2 9 B	1 871	4 85	94	M
			0 744	1 10	37	
			1 547	2 30	77	
	June 4	2 4 A	1 832	3 60	92	
			1 990	4 75	100	
			1 13	1 10	57	
			1 90	2 40	95	
	June 6	2 4 A	2 03	3 60	101	
			2 15	4 75	107	
			0 88	1 10	44	
			1 747	2 40	87	
	June 8	2 9 B	1 961	3 60	98	
			2 057	4 75	104	
			0 905	1 10	45	
			1 534	2 50	77	
3	Oct 20	2 4 A	1 797	4 00	90	M
			1 937	5 50	97	
			1 510	1 50	76	
	Oct 26	2 9 B	1 995	3 10	100	
			2 111	4 50	106	
			2 176	5 75	109	
			1 165	1 5	58	
			1 641	3 0	82	
			1 839	4 5	92	
			2 030	6 0	102	
4	Oct 31	2 4 A	1 828	3 0	91	F
5	Nov 1	2 4 A	1 906	3 0	95	M
	Nov 4	2 9 B	1 887	3 0	94	
6	Nov 3	2 4 A	2 051	3 0	103	M
7	Nov 4	2 4 A	2 00	3 25	100	M
8	Nov 10	2 4 A	1 903	3 0	95	F
9	Nov 9	2 4 A	2 002	3 0	100	F
10*	Nov 27	0 55 A	0 518	3 0	94	F
3†	Oct 29	6 0 A	1 824	1 5	36	M
			4 084	3 0	82	
			4 928	4 5	97	
			5 152	6 0	101	
	Nov 3	7 45 B	2 300	1 5	45	
			3 567	3 0	70	
			4 269	4 5	84	
			4 728	6 0	93	
2†	Sept 5	6 0 A	0 469	0 5	9	M
			1 041	1 0	21	
			2 430	2 0	48	
	Sept 8	7 45 B	4 436	3 5	87	
			5 139	6 0	101	
			0 563	0 5	11	
			1 497	1 0	29	
			2 564	2 0	50	
			3 544	4 0	70	
			4 297	6 2	85	
1	Nov 5	6 0 A	4 728	3 1	93	M
Lewis'		6 0 A	1 746	1 5	34	
experi-			4 345	3 5	85	
ment		8 37 C	4 809	5 0	95	
				3 0	82	

A, sodium benzoate, B, hippuric acid, C, sodium hippurate

* Child 25 years old

† Repetition of Lewis' experiments

TABLE 4—PATHOLOGIC INDIVIDUALS

No	Date, 1920	Substance In-gested, Gm	Hippuric Acid Excreted			Albu-min in Urine	Phenol sul-phone-phthalein Ex-creted in 2 Hours, per Cent	Diagnosis and Remarks		Sex
			Total Calcu-lated as Benzoic Acid, Gm	Total Time Elapsed After Inges-tion of Sub-stance, Hours	Total Excre-tion per Cent of Sub-stance In-gested					
1	Mar 29	2 6 B	0 432	3	24	+	18	Chronic nephritis	Necropsy May 10	M
	April 3	2 4 A	1 041	6	59	+	Mar 15			
			0 557	3	28					
			1 054	6	53					
			1 618	9	81					
			2 070	12	104					
	April 10	2 4 A	3 034	24	152	+	15 April 17			
			0 521	3	26					
			0 965	5 4	48					
			1 375	8	69					
1 610			9 3	81						
2	May 11	2 4 A	0 221	3	11	+	29	Chronic nephritis, cardiac decompensation		M
	May 13	2 9 B	0 857	5 7	43	+	April 4 31 June 6			
			0 318	3	16					
			0 982	6	50					
3	May 4	2 4 A	0 514	3	26			Cardiac decompensation		M
			1 016	6	51					
4	Sept 3	2 4 A	0 542	3	27		28 Sept 8	Aortitis syphilis, cardiac hypertrophy	Necropsy Sept 11	M
	Sept 10	2 9 B	1 073	6	54					
			0 304	2 6	15					
			0 415	6	21					
5	Sept 3	2 4 A	0 866	3	43	+	25 Aug 16	Cardiac decompensation		F
			1 390	6	69					
6	Oct 11	2 4 A	1 324	3	66		50 Oct 9	Cirrhosis, cardiac decompensation	Necropsy Nov 21	M
	Oct 13	2 9 B	0 949	3	48					
	Nov 16	2 4 A	1 270	4 5	64					
7	Sept 30	2 4 A	1 314	3	66		40 Sept 9	Mitral stenosis		M
			2 055	6	103					
8	Nov 13	2 4 A	0 706	3	35	+	43 Nov 11	Cardiac decompensation		M
	Nov 16	2 4 A	1 259	3 9	63					
	Nov 23	2 4 A	1 797	3	90					
	Nov 27	2 4 A	0 964	3	48					
9	Nov 13	2 4 A	1 235	3	62		43 Nov 12	Angina pectoris		M
	Nov 16	2 4 A	1 260	3	63					
10	Nov 16	2 4 A	1 203	3	60		45	Cardiac valvular disease		M
	Nov 20	2 4 A	0 691	3	35					
	Nov 27	2 4 A	1 176	3	59					
11	Nov 13	2 4 A	1 075	3 3	54			Mitral stenosis		F
	Nov 16	2 4 A	1 623	3	81					
	Nov 23	2 4 A	1 601	3	80					
	Nov 27	2 4 A	1 682	3	84					
12	Nov 13	1 2 A*	0 955	3	96			Gastric ulcer		M
	Nov 16	1 2 A†	0 683	3	68					
13	Nov 27	2 4 A	0 193	3	10	+	17 Nov 25	Chronic nephritis	Clinical improvement	M
	Dec 3	2 4 A	0 349	3	17					
	Dec 8	2 4 A	0 438	3	22					
			0 891	6	45					
			1 281	9	64					
			1 582	12	79					
	Dec 16	2 9 B	2 667	24	133	+	16 Dec 16			
			0 396	3	20					
			0 812	6	41					
			1 186	9	59					
			1 559	12	78					
			2 916	24	146					

TABLE 4—PATHOLOGIC INDIVIDUALS—(Continued)

No	Date, 1920	Substance In-gested, Gm	Hippuric Acid Excreted			Albu-min in Urine	Phenol-sul-phthalein Ex-creted in 2 Hours, per Cent	Diagnosis and Remarks		Sex
			Total Calcu-lated as Benzoic Acid, Gm	Total Time Elapsed After Inges-tion of Sub-stance, Hours	Total Excre-tion per Cent of Sub-stance In-gested					
14	Dec 3	2.4 A	0.910	3	45		20	Cardiac decompensation		M
	Dec 7	2.4 A	1.395	3	70		35			
	Dec 10	2.4 A	1.651	3	83		Dec 11			
15	Dec 3	0.8 A	0.469	3	69		70 Dec 10	Postscarlatinal nephritis in stage of recovery	Child 11 years	M
16	Dec 7	2.4 A	0.568	3	28	+	10 Dec 6	Nephritis		M
17	Dec 10	2.4 A	0.812	3	41			Pyloric obstruction		M
18	1921 Jan 10	2.4 A	1.630 3.040	3 24	82 152	+	35 45	Nephrosis† due to abscess		F
19	Jan 9	2.4 A	1.410 2.544	3 24	71 127		52	Pyelocystitis		F
20	Jan 9	2.4 A	1.342	3	67		47	Cardiac decompensation		F
21	Jan 9	2.4 A	1.925 2.542	3 24	96 127		43	Arteriosclerosis		F
22	Jan 10	2.4 A	1.980	3	99			Diabetes mellitus		M
23	Jan 11 Jan 15	2.4 A 2.4 A	0.806 1.026	3 3	40 51		20	Nephritis		M
24	Jan 15	2.4 A	1.419	3	71			Cardiac decompensation		M
25	Jan 15	2.4 A	0.815	3	41			Bradycardia		M
26	Jan 15	2.4 A	1.852	†	93		62	Asthma		
27	Jan 16 Jan 18	1.06 O 1.25 A	0.479 0.994	3 3	45 94			Nephritis, chronic interstitial	Child 11 years old	F
28	Jan 21	2.4 A	0.938	3	47	+		Nephritis		M

A, sodium benzoate, B, hippuric acid, O, benzoic acid

* Benzoate ingested 1 hour before breakfast

† Benzoate ingested with noon meal

‡ See text

Whether the benzoate test can be used as a means of differential diagnosis between the cases primarily cardiac and those primarily renal, our data are at present insufficient to decide, but the results presented in this paper indicate that this is a possibility, and that if several tests are made on the same patient two or three days apart, that with carefully controlled conditions of diet there would be very marked fluctuations in the values obtained with the cases primarily cardiac, but with the nephritics a greater constancy (Cases 1, 8, 10 and 13, Table 4)

Certain advantages which the benzoate test appears to have in comparison with the phenolsulphonephthalein test are (1) Normal values within narrow limits, (2) greater delicacy in picturing kidney function, (3) simplicity in making the test, and (4) excretion of a substance normally eliminated by the kidney

The normal values as we have found them for adults vary from 95 to 100 per cent elimination in three hours These were made on

TABLE 5—NEPHRITIS, BENZOATE TESTS

	Phenolsul phonephthalein Per Cent	Number of Cases Between				
		10-20%	20-30%	30-40%	40-50%	50-100%
Adults	15-30	2	2	0	2	0
Children	53-70	0	0	0	0	2

TABLE 6—CARDIAC CASES BENZOATE TESTS

	Phenolsul phonephthalein Per Cent	Number of Cases Between				
		10-20%	20-40%	40-60%	60-80%	80-100%
Adults	20-47	0	2	4	6	0

members of the faculty of the medical school and students Only one normal result was as low as 91 per cent At present, we have only one figure for the normal output of hippuric acid in three hours for children This was obtained by giving a 2½ year old girl, weighing about 34 pounds, 0.55 gm sodium benzoate or 34/150 of 2.4 on the basis that the latter is the correct dosage for an adult weighing 150 pounds The result obtained in this case was a 94 per cent elimination in three hours Fluctuations in the phenolsulphonephthalein values obtained on normal subjects are enormous With adults 15 per cent or more of the injected dye is not recovered, and its fate is unknown, but Kendall¹⁸ has given a clue to the manner in which this substance may be destroyed in the body It has been our experience that patients object far less to taking a dose of sodium benzoate in solution than to having an intermuscular injection of phenolsulphonephthalein

18 Kendall, E C The Fate of Phenolsulphonephthalein When Injected Into the Animal Organism, J A M A 66 343 (Feb 3) 1917

Of interest are the results of the benzoate test applied to two children (Cases 15 and 27, Table 4) Case 15, an 11 year old boy just recovering from a postscarlatinal nephritis, with a phenolsulphonephthalein test of 70 per cent elimination in two hours, has a benzoate test of 69 per cent, indicating lowered renal efficiency Case 27, an 11 year old girl, with a phenolsulphonephthalein test of 53 per cent in two hours, has a benzoate test of 94 per cent This case was diagnosed as chronic interstitial nephritis on the history of the case, the urinary findings and the blood pressure—systolic 170 and diastolic 130 mm of mercury, respectively In this instance the Mosenthal¹⁹ renal test was applied and found to be nearly normal and more closely agreed with the benzoate test than with the phenolsulphonephthalein test The value of the latter, 53, is lower than the average figure of

TABLE 7—MISCELLANEOUS CASES, BENZOATE TESTS

	Number of Cases	Phenol sulphone phthalein	Benzoate Test
Cirrhosis of liver	2	50 57	66* 94
Gastric ulcer	1		68† 96‡
Pyloric obstruction	1		41
Arteriosclerosis	1	43	96
Diabetes mellitus	1		99
Asthma	1	62	93
Nephrosis	1	46	82
Pyelocystitis	1	52	71

* With cardiac decompensation
† Benzoate given with the noon meal
‡ Benzoate given 1 hour before breakfast

76 per cent, or the lowest normal figure of 64 per cent found by Hill²⁰ in thirty normal children

In Table 7 are shown the results of the benzoate test on patients with diseases not renal in nature Cases of asthma, arteriosclerosis (no clinical signs of kidney involvement), diabetes and gastric ulcer gave normal benzoate test percentages A benzoate test on a patient with cirrhosis of the liver accompanied by cardiac decompensation gave 66 per cent A second case of cirrhosis of the liver showed a normal output of hippuric acid in three hours after ingestion of the customary dose of sodium benzoate, as the figure 94 indicates In neither of these two cases could any free benzoic acid or its salts be found in the urine after the benzoate ingestion

19 Mosenthal, H O Renal Function as Measured by the Elimination of Fluids, Salt and Nitrogen, and the Specific Gravity of the Urine, Arch Int Med **16** 733 (Nov) 1915

20 Hill, L W Mild Chronic Nephritis in Children, J A M A **75** 596 (Aug 28) 1920

Some facts of interest regarding absorption and the possible rôle of this factor in affecting the applicability of the benzoate test are brought out in Cases 12 and 17. In the former, a case of gastric ulcer, a fluoroscopic examination after a barium sulphate meal showed a 10 per cent retention of this at the end of six hours. Two benzoate tests were made, in each case using one-half the usual dose, the first showing a 96 per cent elimination in three hours and the second, 68 per cent. In the first case, the benzoate was given one hour before breakfast, but in the second case it was given with the noon meal. Here the difference is clearly attributable to different rates of absorption, this being at a much slower rate while food was in the stomach.

In Case 17, a nearly complete pyloric obstruction was diagnosed from the fluoroscopic examination after a barium sulphate meal. No passage of this material from the stomach into the duodenum during six hours could be observed. The usual dose, 2.4 gm sodium benzoate was given to this patient immediately after the stomach contents had been removed by means of a stomach tube. The patient was then required to void urine. At the end of three hours, the collected specimen of urine was analyzed and found to contain sufficient hippuric acid to account for an absorption of 41 per cent of the ingested dose of benzoate. From these findings we conclude that such delay in absorption as might possibly be encountered in applying the benzoate test to cases of suspected nephritis would have no effect on the accuracy of the results if the precaution were taken in all cases of this kind to give the benzoate on an empty stomach, preferably one hour before breakfast.

Case 18 was at first diagnosed as nephritis from the urinary findings, although the blood pressure was normal. Two phenolsulphonephthalein tests on December 19 and 29 showed 35 and 45 per cent elimination in two hours, respectively. January 6, blood analysis showed urea nitrogen 12, creatinin 2 and glucose 111 mg, respectively in 100 c c blood. January 10, a sodium benzoate test was made and an elimination of 82 per cent in three hours was noted. This is about 15 per cent under the normal values which we have found, but not so low as any of the results for nephritics or cardiac decompensation cases. At this time a provisional diagnosis of nephrosis due to the absorption of large amounts of toxic substances from an extensive abscess of the buttocks, involving the sacrum and coccyx, was made. It was thought that the presence of this abscess could account for the urinary findings on which the previous diagnosis of nephritis had been made. Due to the pressure of other duties at this time, unfortunately, no more benzoate tests were made. Subsequent data on this case which terminated fatally are as follows:

January 28, the blood urea nitrogen was 43 and the creatinin 42 mg, respectively in 100 c c blood February 9, the blood urea nitrogen had risen to 74 and the creatinin to 12.7 mg per 100 c c blood The necropsy diagnoses a few days later were (1) Acute diffuse glomerular nephritis, (2) streptococcic infection over gluteal and sacral regions

Case 19 was diagnosed as pyelocystitis, bilateral salpingitis and syphilis The benzoate test of 71 per cent indicated lessened functional activity on the part of the kidneys The phenolsulphonephthalein test was 52 per cent elimination in two hours

SUMMARY AND CONCLUSIONS

1 A modification adapting the Folin-Flanders method for the determination of hippuric acid to albuminous urines is described

2 Sodium benzoate, in 2.4 gm doses, is completely synthesized into hippuric acid and eliminated as such in individuals whose kidneys have been demonstrated to have been damaged extensively, these findings having in some cases been checked by necropsy findings In nephritis, hippuric acid is excreted at the same rate whether its source was ingested benzoate or an equivalent amount of hippuric acid in the form of the sodium salt The synthesis must occur fully as fast as the kidney is able to excrete the hippuric acid formed Neither benzoic acid itself nor any salt of it has been found in the urine of any patient so far studied after the ingestion of sodium benzoate

3 The three hour output of hippuric acid on a diet free from fruit and cranberries is relatively too small to affect the results obtained when 2.4 gm sodium benzoate are ingested, and, therefore, has been disregarded in making the benzoate tests From 95 to 100 per cent of the 2.4 gm of ingested sodium benzoate appears in the urine as hippuric acid within three hours, and represents the normal average After ingestion of from 6 to 10 gm sodium benzoate, the rate of elimination is less as shown by Lewis, and Lewis and Karr²¹ Their findings that after the ingestion of an equivalent amount of hippuric acid the rate of excretion is less than after benzoate ingestion has been confirmed by us In repeating one of their experiments we obtained a curve of elimination practically identical with theirs

4 We conclude, that in man the kidney does not play the leading rôle in the synthesis of hippuric acid as has been supposed by various investigators from time to time It may play a minor rôle, for in the normal individual hippuric acid is excreted at a higher rate after ben-

²¹ Lewis, H. B., and Karr, W. G. The Synthesis of Hippuric Acid in the Animal Organism. III. The Excretion of Uric Acid in Man After Ingestion of Sodium Benzoate, *J. Biol. Chem.* **25** 13 (May) 1916

zoate ingestion than after hippuric acid ingestion in equivalent amount, and this difference is also noted in some of the cardiac cases, but not in the advanced nephritics

5 A new renal function test is described in which the ability of the kidney to eliminate hippuric acid at a definite and rapid rate is the criterion Benzoic acid cannot be substituted for the sodium salt in this test, as shown in Case 27, Table 4, for its relatively low solubility in water probably causes a lower rate of absorption At any rate, after its use the amount of hippuric acid eliminated in three hours is about one-half only of that eliminated in the same time after the ingestion of an equivalent amount of sodium benzoate

Our thanks are due to Doctors S Marx White, Moses Barron and M H Hoffman of the University Hospital, to Drs A Stewart, C T Ecklund and L F Badger of the Minneapolis General Hospital, to Drs Rood Taylor and M D Ott of the Abbott Hospital and to Dr M A Shillington for their enthusiastic cooperation in carrying out these tests For helpful discussion of certain pathologic and physiologic aspects of this problem we express our thanks to Drs E T Bell and F H Scott of the Departments of Pathology and Physiology of the medical school

We are indebted to the staffs of the University, Minneapolis General and Abbot Hospitals for the data concerning diagnoses phenolsulphonephthalein tests and blood analyses

Book Review

DIAGNOSTIK DER KINDERKRANKHEITEN MIT BESONDERER
BERÜCKSICHTIGUNG DES SAUGLINGS By E. FEER, Berlin, 1921,
Verlag Von Julius Springer

This volume, consisting of 269 pages, considers in a very interesting and instructive manner the diagnosis of diseases of infancy and childhood, stressing particularly the former. The book essentially considers signs and symptoms. The author finds that this plan of treating diagnostics in infancy and childhood is of the greatest importance, and is also most instructive to the student. It is obvious that, in the case of the infant, the objective symptoms are alone of importance because he has not the power of vocal expression and in somewhat older children the same signs are of equal importance, because the child has not sufficient judgment to interpret and express his subjective feelings.

In his long experience as a teacher, the author has found that it is of great value from the pedagogic standpoint to consider and analyze the outstanding symptoms. Even after a diagnosis has been arrived at, he finds it instructive to reflect on the value of the single symptoms or sign which he has employed to ascertain whether a diagnosis may be established on such a finding. He thinks the discussion of such points as these are of value to the student as well as to the practitioner, and by way of example asks: Is a swelling of the tibia sufficient evidence of late syphilis, does a Chvostek facial phenomena indicate spasmophilia, is a skin eruption of a certain kind sufficiently diagnostic of scarlet fever? The author attempts these discussions in his book. He brings out the points in differential diagnosis, and draws from his long and rich experience in differentiating closely the various signs and symptoms. His conclusions are definite and logical and give illuminating clinical information.

He makes a strong plea for the older and simpler methods of clinical examination. He says that, owing to the great progress which medicine has made during the last decennium, the vast amount of experience and skill which the older clinicians possessed is more or less neglected in the modern clinical examination. He thinks it is an important part of instruction to train the special senses of the examining physician. He insists on this point in his clinic, trains his assistants and students according to this plan. In this way he thinks he has increased their diagnostic acumen and their powers of observation. The patient, himself, is observed and studied by the ordinary clinical methods first, and the aid of the laboratory is sought later to confirm and assist in the diagnosis. If a patient is brought to his clinic for suspected syphilis, the blood is not taken at once and sent to the laboratory for a Wassermann examination, but an exhaustive physical examination is made first. The condition of every organ is carefully noted, every sign and symptom receives careful thought and consideration, and then, after all this has been done, the Wassermann test is made. The procedure is the same for a case of meningitis. Feer does not permit his assistants ordinarily to make a lumbar puncture at the outset, but insists that a detailed history be obtained, and a careful, thorough physical examination be made. After all this has been done the lumbar puncture is performed.

A great number of signs and symptoms are discussed and striking and valuable information is given as to their appearance and importance. In considering physiognomy and facial expression, he refers among other things to the sudden occurrence of pallor. He points out that in a case of bronchopneumonia a striking pallor is ominous, and also that if the normal rosy

complexion of a premature baby suddenly changes to a waxy pallor, and the eyes appear sunken, one should suspect a severe nutritional disturbance or a sepsis. In the same chapter he describes the face which is characteristic of infantile tuberculosis. The upper lip projects upward, the external nares are thickened and often ulcerated and the cheeks are spotted. Very characteristic is the unilateral conjunctivitis, with pericorneal injection, with phlyctenulae and photophobia. There are numerous descriptions of the facial expression of the mongol, of the idiot, and of the hydrocephalic. One of the illustrations accompanying the text shows a four months old baby with wrinkled forehead, which he considers to be a characteristic finding in pyloric stenosis.

In considering the skin eruptions which characterize the acute infectious diseases, he gives careful consideration to diagnosis and differentiation of the common exanthematous diseases. In addition he mentions erythema infectiosum (measles) which is not commonly treated in the textbooks. Of this latter he says that at the onset the eruption consists of small red macules which appear first on the face, and show a characteristic tendency to increase in size and coalesce to form a large red patch. The lesions occur also on the extensor surfaces of the arms and on the glutei.

In discussing the examination of the lungs, he points out that there are certain advantages in auscultating the thorax with the unaided ear, and says that on account of the superficial breathing of young infants the real condition of the breath sounds and the presence of râles is most advantageously elicited if the baby cries.

He presents a very thoughtful chapter on the diagnosis of appendicitis in infancy and young childhood, and in conclusion remarks, that in a doubtful case of appendicitis it would be better to open the abdomen than to wait too long, because while rupture of the appendix would confirm the diagnosis, it would also cost the life of the patient.

The material is well presented. The discussions are concise and one is impressed by the fact that the matter presented represents the experience and thoughtfulness of a master. The illustrations are profuse and well made. We regret, however, that there are not a few colored plates to illustrate the acute infections, and the color changes in the skins and nails.

On the whole, it is a valuable contribution to the clinical study of pediatrics.

EPITHELIOMATA OF THYMIC ORIGIN [†]

DOUGLAS SYMMERS, M D

AND

B M VANCE, M D

NI W YORK

The histogenesis of the thymus is still a subject of debate. Most investigators are agreed that the reticulum and Hassall's corpuscles are epithelial in origin. The derivation of the chief elements of the organ, however, namely, the small cells, has not been determined. For example, Maximow ¹ believes that early in the process of development the thymus becomes invaded by mesenchymal elements which differentiate into lymphocytes and that these accumulate in such numbers as to give to the organ the appearance of a lymphoid structure. Maximow's belief in the lymphocytic nature of the small cells is shared by Hammar ² and Schaffer, ³ but is opposed by Stohr ⁴ and Pappenheimer, ⁵ who regard them as epithelial. It seems to us that the conception of the small thymic cell as a lymphocyte is more in harmony with common knowledge of the pathology of the gland, especially its tumors and, if Maximow's conception is correct, it follows that the parenchyma of the thymus possesses a dual embryogenesis—the lymphoid cells being derived from the mesoderm and the epithelium from the ectoderm, a distinction which the thymus shares with the suprarenal capsule, whose cortical cells are mesodermal, while those of the medulla are ectodermal. Appreciation of the dual origin of the parenchyma of these organs is useful in approaching the study of the tumors that arise from them. For example, in the suprarenal, two clinically and otherwise separable varieties of malignant tumor may be distinguished—one, the so-called suprarenal hypernephroma, the second takes its origin from residual neuroblasts in the medulla, the so-called neuroblastoma, and is consequently ectodermal. Likewise, it is possible broadly to divide the tumors of the thymic parenchyma into two groups—the members of one group springing from the preponderating cell of the adult thymus, namely, the lymphocyte, the members of the other group arising from

[†] From the Pathological Laboratories, Bellevue Hospital.

1 Maximow Arch f mikr Anat **24** 525, 1909

2 Hammar Arch f Anat u Physiol (Anat Abth), 1907

3 Schaffer Sitzbericht kaiserl Akad Wissenach, Wien **118** 1909

4 Stohr Anat Hefte **41** 1910

5 Pappenheimer J M Research **17** 1, 1910

that cell which originally dominated the thymus but which, as development proceeds, is projected into the background, that is to say, the epithelial cell

The thymic lymphosarcomas are divisible into two histologic groups. The first and prevailing type of tumor (a) is made up of lymphoid cells which are arranged diffusely or are gathered in small packets separated from one another by delicate strands of connective tissue. Most of the pure lymphocytic tumors are sparsely vascularized, but in occasional instances blood vessels are present in vast numbers and, in these circumstances, the mass may pulsate, simulating aneurysm. In other cases the lymphocytic growth may persist for a variable length of time, with or without pressure symptoms referable to the upper part of the thorax, and suddenly commence to pour lymphocytes into the circulation in such numbers as to constitute a form of acute leukemia, and thus to terminate life within a few weeks.

(b) The second variety of thymic lymphosarcoma is histologically comparable to Hodgkin's disease and is composed of lymphoid cells among which are giant cells of the myeloid type, together with eosinophils and eosinophilic myelocytes.⁶

To the lymphoid tumors it has been proposed to apply the designation thymoma, but this appellation, it seems to us, is permissible, if at all, only when it is used to denote a tumor that more or less faithfully reproduces the structure of the thymus as a whole, including lymphocytes, epithelial reticulum cells and Hassall's bodies. As far as we have been able to gather, such a tumor has not thus far been differentiated and until it is and until the histogenesis of the thymus is settled, the designation of thymoma must be regarded as misleading and as tending to complicate rather than to clarify the already hopelessly involved nomenclature of the neoplasms. To justify the term thymoma it is not sufficient merely to demonstrate, for example, the presence of Hassall's bodies in the primary growth, but to show that these bodies are newly formed and not residua of the originally normal thymus, and that they occur in association with other structural counterfeits of the thymic tissues. In this connection it is worth while to note that in our search of the literature and in our personal experience with the study of thymus tumors, we have failed to find a case where Hassall's bodies occurred in any of the secondary formations. The growing practice of naming tumors according to the organs from which they spring is a procedure of doubtful value. If the nomenclature of tumors is to be satisfactorily recast, we must seek simpler criteria. For example, hypernephroma means nothing other than a tumor arising somewhere above the kidney, hepatoma conveys no intelligible con-

ception of the cellular genesis of a tumor of the liver, and thymoma does not serve in the least to simplify our knowledge of the more remote origin of the tumors of the thymus. We might more profitably busy ourselves with the task of correlating clinical events with the growth and distribution of tumors and with their cellular derivation as opposed to the organs in which they arise.

In the developing thymus the glandular structures are separated and compressed by infiltrating lymphocytes to such an extent that the



Fig. 1—Low power photomicrograph showing the general architecture of the thymic growth. Above are small cells arranged in alveoli. Below are masses of flattened cells in one of which is a group of small cells.

epithelium is reduced to a combination of delicate reticulum and circumscribed collections of cells known as Hassall's bodies, the latter bearing a resemblance to the epithelial pearls that occur in certain inflammatory lesions of the skin and in many of the epitheliomas of the skin and mucocutaneous junctions. From the observations of Paviot and Gerest, Thiroloix and Debre, and Rubaschow, as well as

from the study here recorded, it seems to be established that there is a variety of tumor of the thymus gland that arises from the epithelial remnants in question, and that the growth shows a tendency to reproduce Hassall's bodies—in other words, that there is a form of epithelioma of thymic origin. Rubaschow divides thymus tumors of this description into two groups—one composed of large flat cells of the epidermal type, the other made up of smaller, rounded epithelial cells, the tumor showing no disposition to develop concentric formations

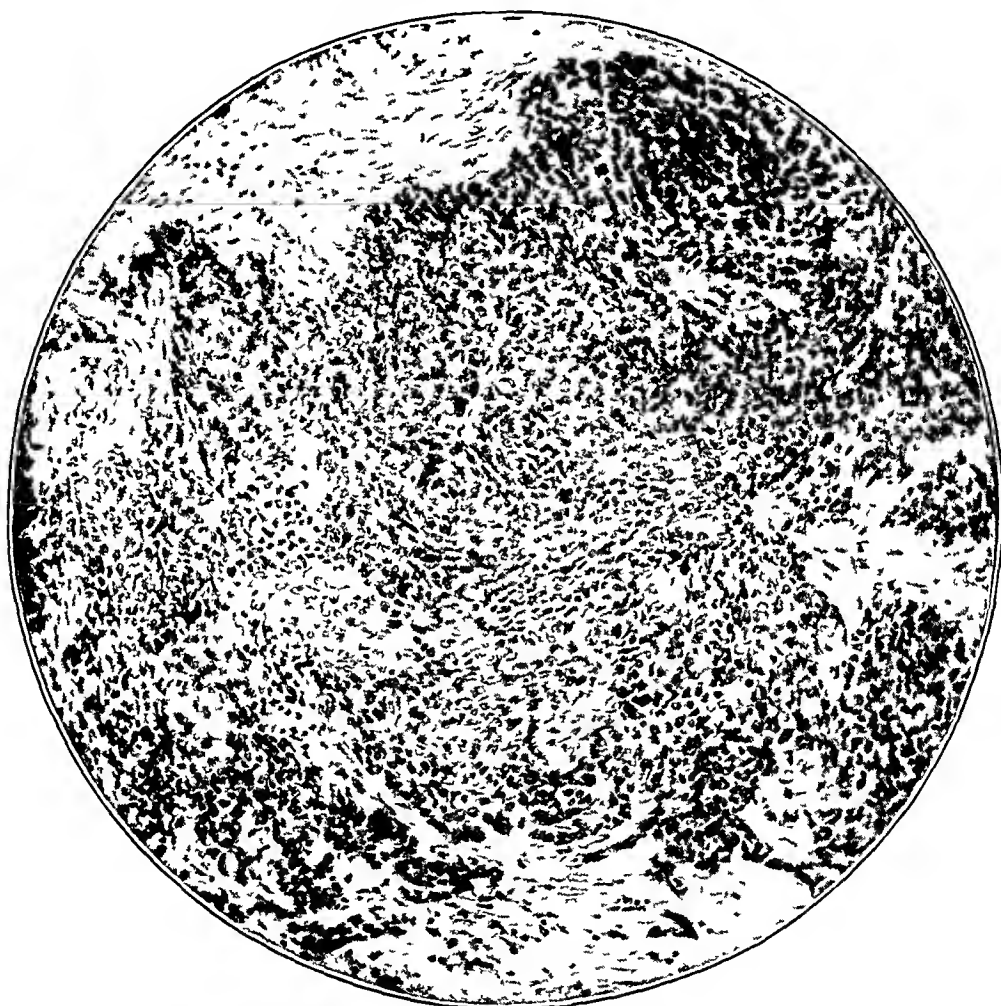


Fig 2—Showing large island of flattened cells. Just to the left of the center is a small whorllike formation.

comparable to Hassall's bodies. In the neoplasm that we describe herein, the histology is rather more complex and partakes of the nature of both the large and small cell types of Rubaschow.

Only three examples of primary thymic epithelioma are to be found in the literature. A fourth is recorded in this paper. Apparently, the first genuine primary epithelioma of the thymus to be described was that of Paviot and Gerest.⁷ The patient was a domestic, aged 52 years

⁷ Paviot & Gerest. *Arch. med. exper.* 8: 699, 1896.

The patient stated that three months before admission to the hospital she began to suffer from retrosternal pains which gradually grew worse and became associated with signs of suffocation and edema of the legs. At the time of admission, the patient was intensely dyspneic and cyanotic, and complained of pains behind the sternum and of cough and expectoration. The voice was of metallic quality. Emaciation was pronounced. At the necropsy, a massive tumor was found

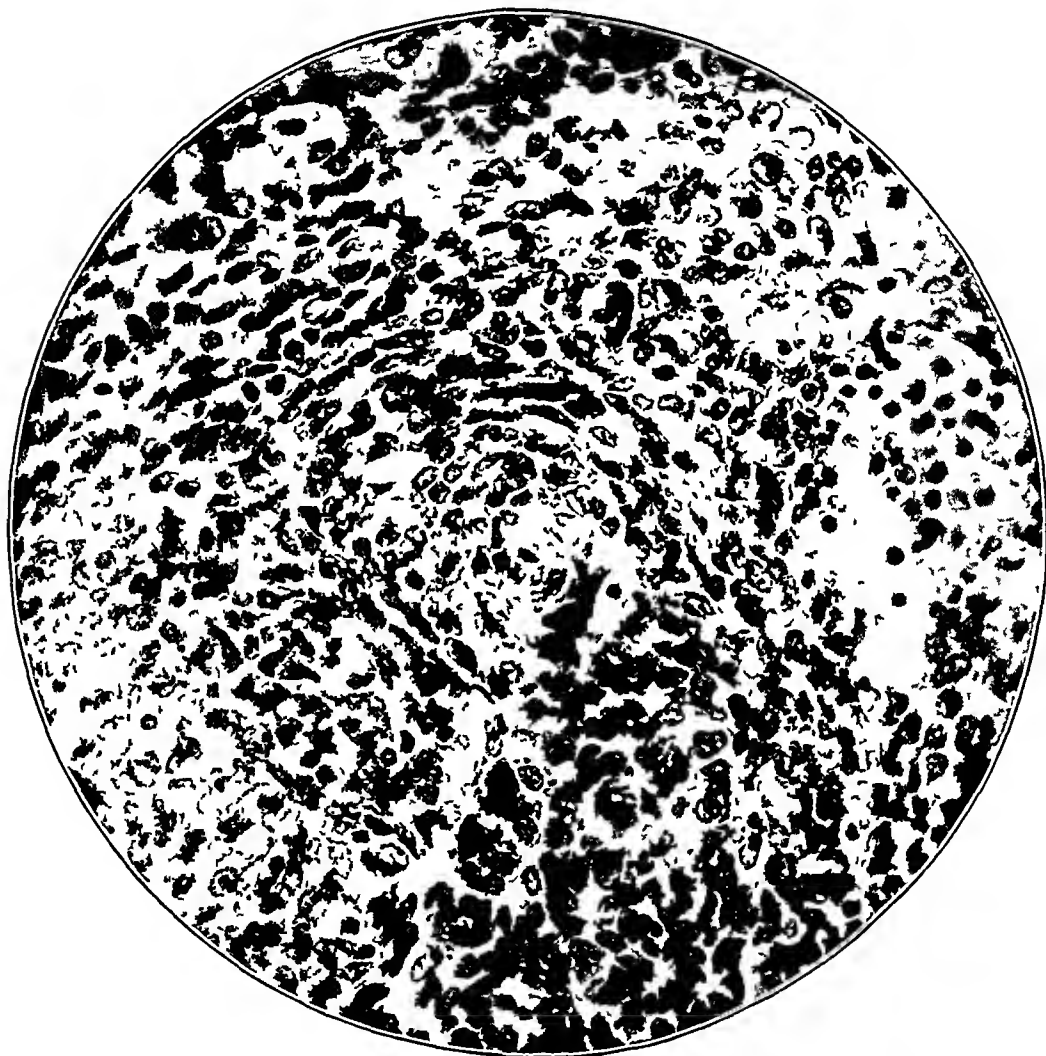


Fig. 3—High power photomicrograph of the whorllike formation represented in Figure 2. Note the shape and arrangement of the cells at the center of the whorl and the general resemblance to Hassall's bodies.

above the heart in the region of the thymus. The growth measured 16x11x13 cm. and was pyramidal in shape, firm in consistence, and the left lung was attached to it. Microscopic examination revealed a neoplasm the cells of which were of different sizes and arranged in unequal strands and columns. The cells were large and some of them were hyperchromatic and of the giant variety. In places the growth showed a tendency to form concentric bodies resembling Hassall's cor-

puscles. The stroma of the tumor was abundant and poorly vascularized. One of the kidneys contained a minute metastatic nodule which, on microscopic examination, was found to possess much the same morphology as that of the parent growth.

The thymic epithelioma described by Thiruloix and Debre⁸ occurred in a man, 56 years of age. The patient complained of retrosternal pains, dyspnea and loss of weight and strength. The total duration of illness was two years. At necropsy, a tumor was found occupying the

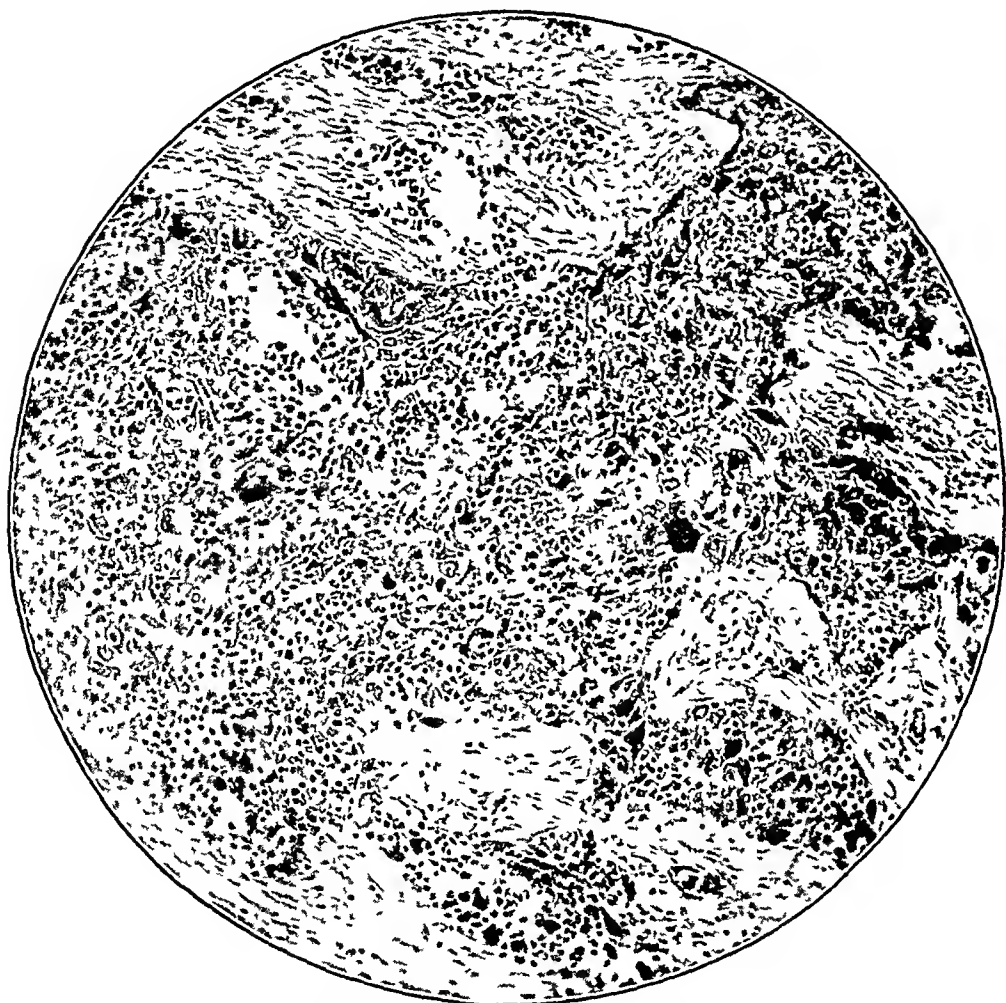


Fig. 4—Field showing extreme variations in the arrangement and morphology of the tumor cells and the tendency to form whorls resembling Hassall's bodies.

thymic area. The growth was roughly triangular in shape with the base upward and was described as about the size of an adult head. It was whitish in color and of cartilaginous consistence, infiltrated the upper borders of the lungs and sternum, and was adherent to the trachea, the pericardium and auricles of the heart, and to the large

⁸ Thiruloix & Debre. *Arch. med. exper.* 19: 668, 1907.

vessels and nerves of the vicinity. No metastases were found. Microscopic examination showed that the tumor cells were from 5 to 30 micromillimeters in size, polyhedral in outline, many of them hyperchromatic. They were arranged in solid cords, showing in places attempts to form concentric bodies with central areas of keratinization. The illustration which accompanies their paper indicates plainly that the tumor belongs in the group of the epitheliomas.



Fig. 5—High power photomicrograph of the hyperchromatic cells shown to the right of the middle in Figure 4. Note the extraordinary variations in the morphology of the cells.

In the case of thymic epithelioma recorded by Rubaschow,⁹ the patient was a man, 62 years of age. During life he presented no signs referable to a growth in the thymic region. At necropsy, a mass was found between the sternum and the pericardium, corresponding to the normal location of the thymus. It measured 12x7x5 cm and was of firm consistence, whitish in color, and adherent to the pericardium,

⁹ Rubaschow. *Virchows Arch f path Anat* 206 141, 1911.

the hilus of both lungs, and the capsule of the thyroid gland. Microscopically, the tumor was found to consist of cells collected into solid groups lying in a supporting network of connective tissue. The cells were rounded or somewhat irregular in shape and noticeably larger than the lymphocyte. Lying among them were remnants of thymic tissue in the form of Hassall's corpuscles.

In the case that we studied at Bellevue Hospital, the patient was a man, 58 years of age, a painter by occupation, who was admitted to

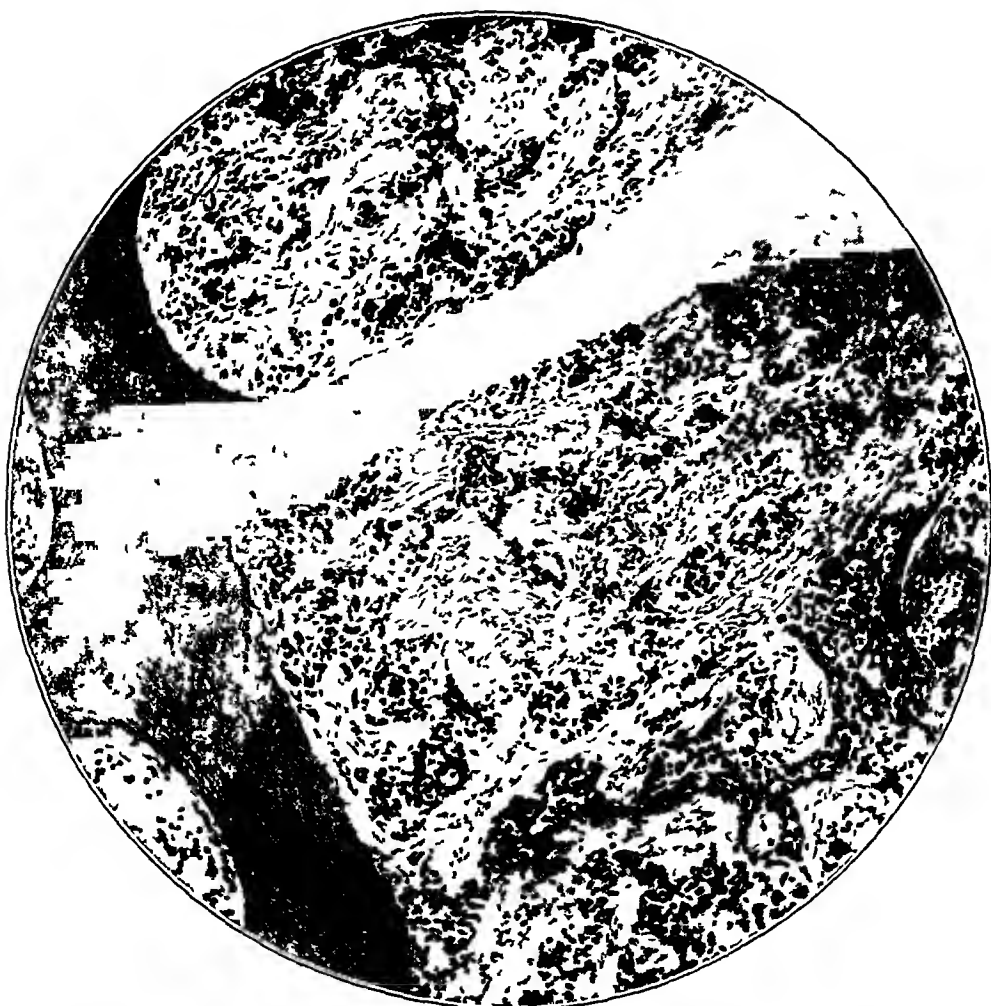


Fig 6—Showing the presence of metastatic deposits in one of the vertebrae (lamina of third dorsal)

the service of Dr. Frank Meara, June 26, and died four months later. The family history was negative and there was nothing of interest in the patient's personal history other than that, ten years previous to admission, he had sustained an attack of plumbism followed by stiffness of the wrist, hip and knee joints that disappeared in the course of the succeeding two months.

At the time of admission the patient complained of severe burning pains in the back that had been present for three weeks. Three days

before admission these pains became so excruciating that he was unable to stand and had to give up work and take to his bed. In addition, he complained of pain in the upper sternal region on swallowing. At the time of admission, it was noticed that the entire dorsal spine was rigid and that this rigidity was increased by movements of the trunk. Two weeks later the patient could not move the body below the waist. The superficial reflexes were abolished, while the knee jerks were preserved, and he was insensible to pain up to a girdle point about 5 cm above the lower border of the ribs. On the following day there were complete paralysis and anaesthesia of the entire body below the level corresponding to the fifth dorsal segment. The patient now complained of no pain except when the head was moved at which time pain was severe and was referred to the region between the fourth and sixth



Fig 7—Marchi preparation from the spinal cord corresponding to the area of compression, showing the mechanical effects of crushing and the widespread degenerative changes in the nerve elements

dorsal vertebrae, where there was also tenderness on pressure. The knee jerks had disappeared and there was a bilateral Babinski. The patient suffered from retention of urine and was incontinent of feces.

The spinal fluid was yellowish in color and contained thirty cells to the cubic millimeter. Examination of the urine revealed nothing of note in the present connection. The Wassermann reaction was negative.

July 16, the spinal column was opened from the fourth cervical to the second dorsal vertebra, and a tumor was removed from the dura mater corresponding to the region between the sixth cervical and the second dorsal vertebra. The growth measured $6 \times 2\frac{1}{2}$ cm and pressed on the dorsal cord. In the succeeding three months the patient gradually failed, bed sores developed and death occurred October 25.

Except for pain on swallowing, the patient at no time during his stay in the hospital exhibited signs or symptoms referable to pressure in the anterior mediastinum

REPORT OF NECROPSY

The body was that of an extremely emaciated man, 58 years of age. In the upper portion of the back, lying in the long axis of the spinal column, was a surgical incision 22 cm in length. Over the sacrum and on the calves of the legs and heels were gangrenous bed sores.

On opening the thorax, a mass came into view corresponding to the position of the thymus. The mass measured $10 \times 8 \times 3$ cm and was roughly triangular in outline, with the base upward. The growth was milk-white in color and of cartilaginous consistence and was attached to the border of the left lung for a distance of about 6 cm. On section, in the center of the tumor, there was a yellowish, cheesy area of softening. Scattered through the left pleura were a few firm nodules varying in size from $\frac{1}{2}$ to 1 cm. The tissue composing these nodules was quite similar to that of the mass in the thymic region. On section, both lungs were studded with nodules of similar description, varying in size from 1 to 2 cm. In some of the larger nodules were yellowish gray areas of degeneration, and others showed grayish white radiating bands of fibrous tissue. The distribution of these nodules was roughly symmetrical throughout both lungs.

The heart was not displaced. The transverse portion of the arch of the aorta passed immediately behind and was partially included in the tumor lying in the thymic region. The aorta itself, however, was not infiltrated.

The laminae of the vertebrae, from the fourth to the eighth dorsal, had been removed. The lamina of the third dorsal vertebra contained dense white tumor tissue lying in the substance of the bone. The spinal cord corresponding to the excised laminae was compressed and there was a wartlike growth, whitish in color, on the posterior and external surface of the dura at about the level of the third dorsal vertebra.

The lungs showed, in addition to the nodular metastases, numerous minute grayish points corresponding to miliary tubercles.

In the small intestine were several ulcers with rounded, undermined edges and fibrous base that varied in size from $\frac{1}{2}$ to $1\frac{1}{2}$ cm.

Anatomic Diagnosis—Malignant tumor of thymus with metastatic nodules in the pleura of the left lung, in the parenchyma of both lungs, in the lamina of the third dorsal vertebra, and in the dura of the dorsal cord, compression of dorsal cord, miliary tuberculosis of lungs, ulcerative tuberculous enteritis, emaciation.

Histologic Examination—The reticulum of the parent tumor in the thymus region consists of rather broad bands of dense poorly staining connective tissue scattered through which are moderate numbers of delicate cigar-shaped nuclei. The fibrous ground substance divides and subdivides in such manner as to segregate the tumor cells into groups which vary markedly both in size and shape. The tumor cells themselves present wide differences in morphology and in their relationship to one another. Perhaps the commonest variety is a rather small cell with a deeply chromatic homogeneous nucleus. Under ordinary magnification, this cell appears to be rounded and of about the size of a lymphocyte, but when viewed under the oil immersion lens with properly diminished illumination it is seen to be irregularly rounded or even polyhedral and to possess a quantity of pale smooth, or finely granular cytoplasm. This appears to be the cell of origin of the tumor and it may be traced through various transitional stages until it acquires a flattened form (Fig 1). These flattened forms give rise to still larger cells of extremely variable morphology. The smaller cells may be seen in abundance scattered through the connective tissue frame-

work as islands or streak-like collections of different shapes and sizes (Fig 1 upper half) In other instances the small cells are found in company with larger, flattened cells which are arranged in solid groups consisting of small oval or rounded collections, sinuous bands, or intercommunicating cords, or as large islands of variable contour (Figs 1 and 2) In the large islands the flattened cells sometimes display a tendency to arrange themselves in small concentric whorls (Figs 2 and 3) In other islands of flattened cells, groups of small cells are to be noted lying in the center or at the periphery (Fig 1, lower half) In still other instances, the islands of flattened cells present marked variations in the shape and size of the individual elements (Fig 4), prominent among which are huge hyperchromatic bodies (Figs 4 and 5), some containing a single nucleus, others a nucleus that is lobulated, still others multiple nuclei In some of these giant cells, evidences of keratinization are to be observed Lying among and around the giant cells are somewhat smaller cells that present almost every conceivable variation in shape A noticeable feature of the large, irregularly shaped cells, as a whole, is that they show attempts to become arranged in whorls and thus to present a certain resemblance to epithelial pearls or to Hassall's bodies (Fig 4)

Microscopic examination of the metastatic deposits in the vertebral column (Fig 6) shows the presence in the marrow of tumor tissue of the same general appearance as that of the parent growth in the thymus, and the same is true of the secondary nodules in the dura, pleura and lungs

Marchi preparations (Fig 7) show degeneration of the entire thickness of the spinal cord at the level where the tumor pressed on it Sections of the cervical cord above the site of the tumor show complete degeneration of the column of Goll, including the cornucommissural bundle, the dorsal and ventral spinocerebellar tracts, the lateral and mesial spinothalamic tracts Below the level of the lesion the lumbar segment shows degeneration in the crossed and direct pyramidal tracts and the scattered fibres of the Deiterospinal tracts The sacral segment shows degeneration in the crossed pyramidal tract and a few fibres in the anterior ground bundle, probably Deiterospinal

Microscopic examination of the other organs of the body showed nothing worthy of record in the present connection

DISCUSSION

From the clinical standpoint, tumors of the thymic parenchyma, both epithelial and lymphocytic, present certain features of practical interest First, the lymphocytic tumors outnumber the epithelial by a considerable margin Moreover, the lymphocytic tumors in the majority of cases occur in individuals under 35 years of age, many of them in children, while all of the few epithelial tumors that have been recorded occurred in persons over 50 years of age

Second, there are considerable variations in the matter of physical signs For example, (a) certain tumors of the thymic parenchyma grow expansively in the upper thorax for long periods of time without giving rise to noteworthy signs of pressure These growths represent a minority, it is true, but that they exist is shown by the complete absence of pressure effects in Rubaschow's case and by the presence of a comparatively trivial complaint in the case that we record, namely pain on swallowing (b) Others grow expansively in the upper thorax and are attended by marked signs of pressure—cough, expectoration, dyspnea, cyanosis, and edema, particularly of the right side of the

chest anteriorly and of the corresponding arm, due to interference with the circulation in the innominate and subclavian veins, hydrothorax, ascites, and the like. A subdivision of this group is represented by those thymic lymphosarcomas which grow expansively for a period of months or years, and suddenly terminate life with the picture of acute leukemia, the tumor pouring lymphocytes into the circulation abruptly and in large numbers—the so-called leukosarcoma of Sternberg¹⁰. As a rule, these growths are attended by pressure symptoms. In one of Sternberg's original cases, however, clinical disturbances are said to have been of only three weeks' duration, yet the intrathoracic tumor was of enormous size and must have been in existence for a period greatly in excess of that suggested by the clinical history. Belonging in this group, also, are those thymic lymphosarcomas which exhibit an extraordinary tendency to bring about symmetrical and diffuse lymphocytic infiltration of the kidneys and massive enlargement of these organs without, however, producing other than slight disturbances of the renal functions. These tumors are usually attended by symptoms of intrathoracic pressure, although in two of Symmers' cases¹¹ of thymic lymphosarcomas clinical symptoms were present for only three and two weeks, respectively, while in both instances the primary tumors, at necropsy, were found to be immense, so that their origin must have antedated the onset of symptoms by many months.

From these facts it is apparent that primary thymic tumors, whether of the lymphocytic or epithelial type, are inconstant in producing signs of intrathoracic pressure—some of them appear to infiltrate so insidiously that the structures of the mediastinum are enabled to accommodate themselves to the process of invasion, and pressure symptoms, if they arise at all, are negligible in degree, others gradually attain enormous proportions and, suddenly and for no apparent reason, become associated with signs of intrathoracic pressure, followed in a few weeks by death, while still others are marked by pressure signs that arise early and are persistent and most distressing. Finally, primary tumors of the thymus, as exemplified by the case we have described in this paper, may give rise to comparatively mild signs of intrathoracic compression, but, by metastasis may produce terrible destruction in adjacent extrathoracic tissues.

From the standpoint of malignancy, it is important to observe that the lymphosarcomas and epitheliomas of the thymus gland do not differ essentially from tumors of the same sort occurring in other localities. Wherever it arises the lymphosarcoma is eminently a growth of regional distribution although, as mentioned, it occasionally displays

10 Sternberg *Beitr z path Anat u z allg Path* **61** 75, 1916

11 Symmers *Arch Int Med* **22** 237 (Aug) 1918

a faculty for bringing about lymphocytic invasion of the blood stream and certain viscera, particularly the kidneys. At other times, it is associated with lymphoid nodules in such organs as the liver, kidney and suprarenal capsule, where, in normal conditions, lymphoid cells are present only in the form of foci so minute as scarcely to attract notice even on microscopic examination. To the unaided eye these nodular "metastases" of lymphosarcomas appear to be well circumscribed but, on microscopic examination, they fade into the surrounding tissues in such graceful fashion as to suggest hyperplasia of pre-existing lymphoid dépôts rather than newly formed foci developing from transplanted cells.

In the same way, the epitheliomas of the skin and mucocutaneous junctions commonly exhibit a tendency to remain localized—that is to say, to infiltrate neighboring tissues rather than to metastasize to distant parts. That they do at times become widely disseminated is not, of course, to be denied, but in these circumstances metastasis is apt to occur as a late event.

These facts are of moment as applied to the lymphosarcomas and epitheliomas of the thymus gland, our present knowledge of which is based practically exclusively on evidence derived from the completion of neoplastic growth as revealed by examination of the body after death. As the attention of physicians becomes more and more focused on the early diagnosis of tumors of the thymus gland, it is not too much to hope, perhaps, that at least some of these growths may be discovered before neighboring tissues have been irreparably damaged. The preponderance of lymphoid growths of the thymus and the known effects of radiation on lymphocytic tissues suggest that the use of the roentgen ray might be beneficial in the treatment of tumors of this type. Also it is conceivable that greater attention to the interpretation of symptoms of pressure in the anterior mediastinum, coupled with such diagnostic aids as the roentgen ray, might sometimes lead to detection of thymic tumors sufficiently early to permit of their enucleation before they have progressed too far.

BETELNUT CHEWING AND ITS EFFECTS, INCLUDING CANCER OF THE MOUTH

ALLER G ELLIS, M D
BANGKOK, SIAM

Medical literature contains many references to cancer of the mouth in betelnut chewers. This sequence of events is cited in illustration of malignant growth developing as a result of chronic irritation (injury) of tissue. Statements regarding this subject are based largely on observations made in India, Africa and some of the islands in the Pacific Ocean. The receipt in the pathological laboratory of Siriraj Hospital of specimens of cancer of the lip from persons using betelnut, suggested an investigation of this question in Siam where the habit is exceedingly prevalent. It was soon found that the scarcity of systematic records in this country makes an extended statistical study of cancer impossible, but, as in other countries, very much that cannot be reduced to exact figures may be learned. The subject was accordingly taken up in a somewhat broader way, and this promptly led to the realization that another of its phases is of more practical importance than is its relation to cancer.

As a part of this study, I sent a questionnaire to twenty-five physicians, including those at the various Mission stations, and the replies thereto represent all parts of the kingdom. Much of the data contained in this paper was furnished by these colleagues, for whose aid I am very grateful.

Except in the larger cities, nearly all adult Siamese chew betelnut. Exceptions in the cities occur among those who have been in foreign countries or who have attended advanced Siamese or foreign-taught schools. In some of the provinces there is said to be a decrease in the habit in the younger generation, but this is not in all instances clearly demonstrable, in Bangkok it apparently is true. It is said that habitual users in the south chew much greater quantities than do those in northern Siam. Chewing is more nearly universal among women than among men and, in general, women chew more of the substance. Some of the large numbers of Chinese here are addicted but the majority are not, among them, women chewers are less numerous than are men.

Betelnut chewing, as practised here, is a very complex procedure. In making up what tobacco devotees call a "chew," people use many substances. Of these at least four are of recognized medicinal value, thus contributing to the medical feature of the subject.

A—THE INGREDIENTS EMPLOYED

1 The leaves of *Chavica betel* or *Piper betel*, commonly called betel leaf (colloquially, plue or ceri leaf) These are glossy green, ovate leaves, from 3 to 5 inches long and from 2 to 4 inches wide, they have a burning, aromatic, bitter taste They are obtained from a vine that is grown on plantations in the shade of small trees planted for that purpose The vine is sturdy and grows to from 8 to 12 feet tall The leaves are ready for use when the vine is 1 year old, and are gathered as they attain proper size for about four years when the plant has to be replaced

Writers differ as to the essential principles of the leaf Khory and Katrak¹ say that it contains betel oil and chavicol, the latter obtained by treating betel oil with caustic alkali The juice of the leaf, they say, is a stimulant, carminative and antiseptic It is given in cases of flatulence, fetor of the mouth, dyspepsia and colic They claim that chavicol is a powerful antiseptic, five times stronger than phenol

Kirtikar and Basu² say the leaves are used in India for many purposes, as the juice is aromatic, carminative, stimulant and astringent, and is regarded as a valuable stomachic For this reason, ancient Hindu writers recommended taking it "early in the morning, after the morning, after meals and at bedtime" In catarrhal and pulmonary affections, the leaves are smeared with oil, warmed and applied over the chest to relieve cough and dyspnea These writers state that recent analyses by workers in India revealed phenols and terpene-like bodies but failed to detect chavicol

2 The nutlike seed of *Areca catechu*, commonly called the betelnut palm The only reason I have found for giving it the latter name is that the nut is chewed with the betel leaf, and from this has acquired the name of betelnut and the tree that of betelnut palm Literally, there is no such thing as betel "nut" but the name is so common that it would be unwise to attempt to change it I use the term betel many times in this paper as a shorter word but not with the idea of supplanting the older name The fruit is nearly two inches in diameter, with a thick green husk and orange colored kernel This ingredient, including the kernel and the inner layer of the covering, is preferably used fresh, but much of it is preserved by opening and drying

The kernels are said to contain catechu, tannic and gallic acids, oily matter, gum and several alkaloids, the chief of which is arecoline Khory and Katrak say the fresh nuts are intoxicating and cause giddiness (That this is the effect on beginning chewers is common knowledge) Dried ones are a gentle stimulant, astringent and tenifuge

1 *Materia Medica of India*, 1903

2 *Indian Medicinal Plants*, 1918

They increase the flow of saliva, lessen perspiration, sweeten the breath, strengthen the gums, remove bad tastes from the mouth and produce mild exhilaration. They are recommended in cases of intestinal worms and dysentery. In its action arecoline resembles pelletierine or pilocarpin, as a myotic it resembles physostigmin. The powder obtained by calcining the nut is known as areca charcoal and is used as an ingredient of a tooth powder.

Kirtikai and Basu say that chewing the dried nut produces a stimulating and exhilarating effect. They quote Dr. Waring as stating that the claim of its being an anthelmintic is hardly to be supported because intestinal worms (*lumbrici*) are almost universally present in Hindus and Burmese who chew it. In Borneo the fragrant flowers of the tree are mixed with medicines and used as charms for the cure of many diseases.

3 Black catechu or cutch. This is the extract of the wood of *Acacia catechu* (Siamese sisiet tree). Dey³ describes it as an astringent and tonic of value in diarrhea, sponginess of the gums and mercurial salivation. It is said to be more powerful than pale catechu from *Uncaria gambier*. This substance appears to be used in the northern part of Siam more than in the south.

4 Root of *Cucuma aromatica* or wild turmeric. This plant is said to contain a volatile oil, resin, starch, sugar, gum and curcumin, a yellow coloring matter. It is used in medicine as a stomachic, stimulant, carminative and tonic. As a masticatory, the powdered root is mixed with lime (paragraph 5) and enough water to make a paste which becomes red in color, possibly due to oxidation.

5 Lime.—This is of the ordinary slaked variety (limestone) or from shells. The use of one or the other apparently depends largely on locality. This substance is mixed with the turmeric and water to form a thick red paste, the usual way, or by some it is used as a powder without the turmeric. The lime is used to counteract the "hot" and astringent taste of the betel leaf.

6 Tobacco.—This is raised in northern Siam. "The leaf is of a peculiarly fine texture. A large portion is used for chewing, mixed with the areca nut and betel leaf. Foreign tobacco is never used in this way."⁴

7 Minor ingredients, such as camphor, cloves, sen-sen, tea leaves, and chestnut tree bark are added by some persons according to their taste. The use of such substances varies to some extent in different parts of the country and appears to be partly a question of local custom.

³ Indigenous Drugs of India, 1896.

⁴ Carter. The Kingdom of Siam, 1904.

The chief or staple ingredients, therefore, are betel leaf, areca nut, black catechu, lime-turmeric paste and tobacco. Four of these without including tobacco, have medicinal qualities. In physiologic action three of them are astringent. Other attributes possessed by or claimed for the substances collectively are those of carminative, tonic, stomachic, antiseptic, sialagogue, antidiaphoretic vermifuge and dentifrice. The effect of the mixture when chewed is moderately stimulating and exhilarating. The effect on the beginner is something like that of using tobacco the first time, giddiness and faintness occur but not nausea.

B—METHOD OF USING

This varies considerably. When lime paste is used, a small quantity is spread on the central portion of a fresh green betel leaf. To this are added the other ingredients, or certain ones only, or none at all. The leaf is then rolled into an elongated cone, about three inches in length and less than one-half inch in diameter at the large end, if the areca nut has not been included. The prepared leaf is then taken into the mouth, followed by the areca nut or tobacco or catechu or all these, and any other desired substance that has not been wrapped in the leaf. Older people without teeth often put all the ingredients into a tube with a movable bottom and mix or rather tamp them together with a plunger. The mass is then pushed out at the top and taken into the mouth.

Actual "chewing" varies with different persons much as it does with tobacco users. Some chew actively and use from fifteen to thirty preparations per day, exceptional cases of persons who use from fifty to eighty preparations daily are reported. Other users are more moderate, the mass often being held for some time between the teeth and the lower lip, either in the midline or toward one angle of the mouth, some keep it more within the cheek. Most people expectorate the large quantity of saliva that collects with the juice, the mixture being a fluid of deep brownish-red color. (On clothing this makes a stain that is removed with great difficulty.) Some persons, especially those who chew unostentatiously, like some users of tobacco, swallow a part of the saliva.

C—LOCAL EFFECTS OF BETELNUT CHEWING

In the habitual chewer, the lips and tongue and, to a lesser degree, the mucosa of the cheeks are colored brownish-red. Castellani and Chalmers⁵ speak of a condition that might be called red or purple tongue which may be puzzling to a medical man newly arrived in the East until he finds that it is due to chewing betel. They say the pigmentation slowly disappears when the natives discontinue chewing

⁵ Manual of Tropical Medicine, 1919

The tongue may remain smooth but often it becomes very dark in color and the margins dry and rough or even cracked. I have seen a considerably roughened tongue in a girl, aged 13 years, who had been chewing for only one year. The lips of some chewers become very rough. It is not uncommon to see women with the mucosa of the lower lip (which may be thickened and everted) entirely denuded and roughened by quite deep furrows. Such mucosae are dry rather than inflamed and exudative. The upper lip is affected to a much lesser degree. The entire mucosa of the mouth undergoes a more or less marked change which may, perhaps, appropriately be described as tanning.

The teeth become very dark brown or almost black in color. This is due to a deposit of the colored lime (so-called calcium concretion) which collects on the teeth. This begins on or near the extremity of the tooth and finally covers over the entire crown, unless it be the points of cusps. Actual "staining" of the teeth does not seem to occur, at least in those teeth which I have examined the enamel is intact and perfectly white when the concretion is removed. As a rule, the concretion is very closely adherent and has to be removed with a knife. On some teeth the deposit has a dull, rough surface, but on others, or on parts of them, it is smooth and has a shining luster like polished ebony. The thickness of this deposit varies. On the biting surfaces it is usually quite thin if the teeth are in actual use. If they do not meet because the opposing ones are out or are loose, it may become very thick. Occasional teeth are found with a deposit on the end thicker than the length of the crown itself.

On the sides of the teeth, the deposit, in many instances, becomes quite thick. Occasionally, the space between contiguous teeth is filled in, converting two or more into a solid mass. If the gum separates from the neck of the teeth and they become loose, the deposition continues downward on the root, as it is gradually exposed by atrophy of the alveolar processes and consequent lessening in depth of the socket. In this way, it finally covers a large part of the root. Measurements of a few teeth extracted when almost loose enough to fall out will give an idea of the surprisingly short portion of some roots left uncovered by concretion. The first number given is the length of the entire tooth, the second number is the length of the uncovered portion of the root from the lower edge of the concretion to the tip. A 20 mm, 5 mm, B 28 mm, 6 mm, C 27 mm, 2 mm, D 25 mm, 10 mm, E 18 mm, 3 mm. (See illustration). All these are teeth with single roots, incisors and canines. One molar has a concretion covering the external surface of one root to its very tip and forming a mass 5 mm broad at the extremity. The other two roots are about half covered, the entire crown is encased. The larger part of this particular concretion is gray in color.

The lower border of the concretion on many teeth, like those described, or even when it extends only to the neck or slightly below, is rough. Some extending downward on the roots taper to almost knife-like thinness. Most of the latter for the lower few millimeters of their length are 1 or 2 mm distant from the tooth. Just what was in this space between tooth and concretion while the latter was being formed is not entirely clear, the opinion of dentists is that it was a portion of the receding gum which had kept close to the tooth as it was carried downward by atrophy of the alveolar process beneath and pressure by the concretion above. I am unable either to verify or



Teeth (from different persons) which became loose and were extracted. Those in the upper rows with the cusps showing white had opposing teeth, those with entirely dark crowns, as in the lower row, had no opposing teeth and show very thick deposit on the ends as well as on the sides. Note the very short portion of some roots left uncovered by concretion. Teeth like those on the extreme right and the second tooth from the left in the lower row lend weight to the view that concretions press down the gum. The tooth on the extreme right of the middle row and the tooth on the left in the lower row have spaces between the concretion and the root, this not being well shown in a side view.

The light mark on the fourth tooth in the middle row was made in an attempt to saw the tooth lengthwise, the enamel under the concretion proved to be harder than the saw. The concretion on the second tooth from the left in the middle row is the only one not colored, it looks like fine gray limestone. From the light areas on the third and fourth teeth in the upper row, the concretion was scraped away with a knife.

disprove this. These projecting rough concretions must be a continual source of irritation to the surrounding tissue. They explain very definitely the statement of one physician that it is impossible to cure pyorrhea and clear up foul gums until these concretions have been removed.

The unanimous statement of physicians and dentists (and the laity) is that betel chewing prevents decay of the teeth. This is abundantly substantiated by the conditions found. One says "Actual decay of the teeth is practically unknown among these people." The covering of the teeth by the dense, polished, intimately adherent concretions apparently protects them. Whether the action of the chewed substances themselves on teeth unprotected by deposit prevents caries is difficult to say because such teeth in chewers are not numerous. Some believe that this is true, and that moderate chewing without deposit is beneficial to the teeth. I do not know that this has been demonstrated in such a way as to warrant the positive statement. However this may be, the statement can be made that so far as caries is concerned, betel chewing, as popularly indulged in, is a preservative of the teeth. I believe this is the only favorable statement that can be made about it. If used to a limited degree as a dentifrice only, with frequent removal of the lime from the teeth, the gums and teeth might be benefited, although I cannot conceive of any one using it for this purpose alone.

Opposed to this preservative action as regards caries is the fact that the loss of sound teeth is very common among betel users. That pyorrhea is prominently implicated in this is indicated by these statements in my reports: "Pyorrhea is very prevalent, the teeth are usually loosened", "pyorrhea practically universal", "teeth are loosened by pyorrhea and retraction of gums", "very little decay, very 'much loosening'", "chronic condition of pus discharging sockets for years", "dental caries very rare. Loosening of the teeth very common", "a native may have lost all his teeth by 50 years of age or less", "little or no decay of the teeth. The gums receding the teeth project and may be removed with the fingers", "a distinct preservative from decay, even if a tooth is badly broken, but teeth are loosened by pyorrhea and retraction of gums", "many people at 40 are quite devoid of teeth."

Some of these statements are not as definite as could be desired, but the only conclusion to be drawn from them as regards pyorrhea is that this condition is exceedingly common. Quite dissimilar to this are the findings of Dr. H. R. Day, dentist at the Chulalongkorn Hospital, who states that of the many loose teeth extracted by him, not more than 10 per cent exhibit pyorrhea. For this discrepancy in findings I think there can be invoked at least three reasons: (1) The well known difference of opinion among physicians and dentists as to exactly what constitutes pyorrhea alveolaris. How much separation of gums, how much pus, how much looseness of teeth, etc., must be present as a basis of diagnosis? (2) Lack in many cases of special examination of persons' mouths with the points raised in this paper definitely in mind. (3) The possibility that in some instances of very loose teeth with very

shallow root sockets due to atrophy of the alveolar processes, pyorrhea may have been present earlier and disappeared as more thorough drainage was established by this lessening in depth. For this reason, I do not believe that the question of pyorrhea can be settled by a study of these very loose teeth. This view has been further strengthened by my observation of earlier cases, although relatively few in number. Of twenty-seven persons whom I have examined, six had distinct pyorrhea and eight more a condition that might by some be termed an early stage of that process, but which conservatively had best be excluded. Four of the six who had pyorrhea, and five of those who did not, had one or more loose teeth, all of the latter series were in those with possibly early pyorrhea.

The importance of this point is not whether pyorrhea loosens teeth, because it does, but what percentage of the teeth becoming loose in betelnut chewers is the result of pyorrhea, and what percentage is the result of other factors strongly emphasized by some physicians and dentists? Among these factors are (1) The mechanical pressure of concretions on the gums and alveolar processes, causing the latter to atrophy and the former correspondingly to recede. According to this view, the sockets progressively become shallower and the teeth become loose because they are deprived of support. (2) Shifting of the line of pressure by opposing teeth from vertical to diagonal because of concretions forming unequally on them. The direction of this secondary line of pressure varies from time to time as the concretion is building, and finally the tooth is loosened.

The limited number of cases I have studied has given me the impression that loosening of the gums from the teeth and infection between the two is quite common before concretions are large enough to cause notable pressure. In occasional cases, there has been shrinking and separation of the gums when the deposit extended only half way from the extremity of the tooth to the gum. In those cases in which there is a thin layer of deposit extending beneath the level of the gum, the inference must be that separation of the gum first occurred or the deposit could not be between it and the tooth. In this connection the statement of Tomes⁶ made years ago is pertinent. In discussing the relation of tartar to pyorrhea alveolaris (of course, in people not chewing betelnut) he says, "One of the earliest indications of the advent of this state of things is a thickening and rounding of the edge of the gum, which ceases to be closely adherent to the neck of the tooth." He states that the dark tartar found between the edges of the gums and the teeth could not have gotten there until the gum had begun to peel away from the necks of the teeth. I have received

6 Dental Surgery, 1887

this impression regarding the thin lime concretions seen in many cases of loose gums. However, it has not been my good fortune to see advanced cases with teeth hanging only by the tip, and with massive concretions like those shown in the accompanying illustration. Possibly, in those cases the mechanical feature of pressure would appear much more prominent.

I believe that the exact mechanism of loosening of the teeth (the same as the incidence of pyorrhea) cannot be determined from a study of advanced cases alone. These questions as to the frequency and effect of pyorrhea and the other possible factors in loosening the teeth can be solved only by long and careful study of many cases of various degrees (especially the early cases) of involvement, including repeated examination of the same persons at intervals of months or years. Now we can only say that a large percentage of betel chewers lose their teeth, but that the mechanism by which this loss occurs is not well understood.

The loosening and loss of teeth, from the standpoint of digestion and nutrition, is serious, and if this result of betel chewing can be lessened or prevented, the question is of great practical interest to any country whose people indulge in the habit. Cleaning of the teeth, the same as for those who do not chew, would seem to be of prime importance. I am informed that those who do this thoroughly, with certain preparations, do not so surely lose their teeth. This is a point to be investigated by physicians, and recommendations should be made to users. If the separation of gums from the teeth by the astringent action of the substances used is a primary effect, the way is paved for infection or for absorption of periodontal structures, and simple cleansing in itself cannot be of great efficacy in saving teeth. Possibly, some other prophylactic may be of use. If, on the other hand, the loosening is a mechanical process in which the deposit of concretions is the primary factor, frequent and thorough cleansing, to prevent the accumulation of lime, would naturally be the indication. A comprehensive study of the whole subject appears very desirable.

C—CONSTITUTIONAL EFFECTS

As to digestive or other constitutional effects of betel chewing per se, most observers agree that these are slight. A few observers speak of fairly common digestive disturbances among the Siamese people (in the opinion of one—starch indigestion), and of a suspicion that this is an indirect effect of the substances chewed or of the saliva that is swallowed. (It would be interesting to note whether this occurred especially in persons with loose and tender teeth. Constipation as an effect is mentioned by one observer only, some say this is a noteworthy condition only in old people who do not take exercise. Dr R W Mendelson, acting medical officer of health of Bangkok, states that the

observation of more than 40,000 patients in their hospital clinic has led him to believe that betel chewing has no untoward digestive effect, his findings in the gastro-intestinal tract postmortem have confirmed that belief. My own experience in nearly 200 postmortems here has led to a similar conclusion. Although no microscopic study has been made of the stomach and intestines in all cases, the gross appearances certainly have not been suggestive of disease. Of course, these negative findings would not exclude the possibility of clinical manifestations.

Rho⁷ says that the use and misuse of the elements employed in chewing may appear unappetizing, but damaging effects do not follow. Castellani and Chalmers state that in the young the habit may possibly be the cause of diseases of the heart and nervous system. Most of my correspondents say that their observations have not led them to believe that betelnut chewing causes any untoward digestive or nervous or circulatory symptoms.

As to the general effect on the body of pyorrhea as an infection, much might be said. I do not intend to discuss that problem in any detail. However, it is an infection, and an infection at any point in the body can exert an influence elsewhere. One physician writes: "There is much so-called rheumatism in this locality. Some of it is without doubt gonorrheal arthritis. I am convinced, however, that more than half the number of cases are from other causes, and the pus foci around the loosened teeth are open to suspicion." Whatever may be said, in general, about pyorrhea alveolaris applies to that condition in betel chewers.

D—THE BACTERIOLOGY OF BETELNUT CHEWING

This study early led me to note the discrepancy between the claim of antiseptic properties for the betelnut mixture and the condition of the mouths of users as described by my correspondents and seen by myself. Theoretically, the mixture employed should be, at least, mildly antiseptic, practically, foulness of the mouth and suppuration are very common. This suggested a bacteriologic study of the mouths of betel chewers. With the permission of Director Luang Ayura Bhatt, examinations were made of twenty patients in the medical, surgical and obstetric wards of Siriraj hospital. The results were so uniform, that it seemed unnecessary to examine a greater number to determine what we wished to know. In this work I was ably aided by Khun Traikisayanukara and Nai Thieb, and it is a pleasure here to acknowledge their help.

The study included a general inspection of the mouths and gums and teeth for decay and looseness. Inoculations were made from the

⁷ Mense. *Tropenkrankheiten*, 1914

margin of the gums between the teeth or from pockets around loose teeth. Spreads were also made for staining, and in fifteen of the cases enough exudate was obtained from the gums or around the teeth to make fresh specimens for microscopic study. From six of the persons a specimen of the red colored saliva was obtained in sterile Petri dishes.

The results of these examinations may be summarized thus:

Persons examined, 20

Sex Women, 15, men, 5

Age From 13 to 54 years

Condition of mouth (gums) Clean, 5, moderately foul, 6, very foul, 9

Lower lip Cracked, rough and dry, 3

Gums Shrunken (contracted) to varying degrees, 20, loose from one or more teeth, varying degrees, 17

Teeth Entirely black, 19, bases not black, 1, one or more loose, 9, decayed, one molar

Pyorrhea alveolaris of various degrees, 6, probably beginning, 8

Amebae (*Endameba gingivalis*) In 9 of 15 fresh specimens

Polynuclear leukocyte exudate around teeth, 17

Bacteria 1 Spreads from gums, cocci in 20, bacilli in 20, spirochetes in 20, fungi in 1

2 Inoculations from gums, positive in 20, cocci in pure culture in 6, cocci and bacilli in 14, streptococcus in 1, pneumococcus in 1, cocci predominant in 12. Dominant coccus, *Staphylococcus pyogenes aureus*

3 Saliva (six specimens) Growth from 6, cocci in 3, cocci and bacilli in 3

From this it will be seen that the mouths of betelnut chewers possess a rich bacterial flora, not exceeded by those (even with pyorrhea) who do not chew. No special attempt was made to isolate and identify the various organisms because an intensive study of the varieties was not an aim. One object was to determine if bacteria, spirochetes and amebas, thrive in the mouth in the presence of the substances chewed and kept within it almost constantly by the users of betelnut. This surely can be answered in the affirmative. Cocci were present, often in great numbers, in every case. The bacilli, also present in every case, were of various types, including some of the large nonpathogenic forms. Spirochetes (*S. dentium* and *S. buccalis*) were present in every case, often in tremendous numbers. Amebas were present in more than one-half the specimens examined for them. These findings do not indicate antiseptic properties of betelnut. A second point of inquiry is really only a variation of the first, namely, has the red colored saliva of the chewer any antiseptic or bactericidal properties. As the saliva and juice collect in the mouth, the mixture is supposedly contaminated by the bacteria on the gums and around the teeth. This supposition was shown to be correct by the abundant growth obtained from inoculations of all six specimens.

In connection with the sixth specimen, the question arose as to what would be the effect on the contained bacteria of continued presence in this juice. Inoculations were made from it after the lapse of twenty-

four, seventy-two and ninety-six hours. Each culture gave an abundant growth of cocci and bacilli, the same as developed from inoculation immediately after the specimen was obtained. This showed that these bacteria had lived in the mixture for four days, apparently with undiminished powers of growth. During this time, evaporation had occurred, and at the end of the ninety-six hours the specimen was only a mass of dry reddish granules. This result indicated that the betelnut mixture possessed no bactericidal properties. To support this conclusion, saliva was obtained from five other chewers. Each gave a growth of cocci and bacilli when obtained and also twenty-four and forty-eight hours thereafter. Two cultures had then developed molds and could not be used further, but the remaining three cultures gave a similar bacterial growth from inoculations made after the lapse of ninety-six hours.

To still further test the betelnut, a "chew" was purchased and macerated in a sterile mortar. Inoculations from the expressed juice remained sterile. Enough sterile water was added to make a thick red fluid and some of this was poured over an agar slant and some into a tube of bouillon. Both were proved sterile and then the agar slant was inoculated from one of the specimens of saliva, in twenty-four hours the red surface showed a heavy growth of bacteria. This betelnut-agar tube was kept for one week, part of the time in the incubator, part of the time at room temperature, and then an inoculation was made from it, that the bacteria were still living was shown by an abundant growth. A spore forming bacillus had also developed at one point.

The tube of bouillon colored red by the betel juice was inoculated, and in twenty-four hours was made turbid by a growth of bacteria similar to those placed in it. The turbidity increased the following two days, and six days later the bouillon gave a positive culture, proving that the bacteria had lived that long in the betelnut bouillon.

From these studies of persons' mouths and of betelnut, we reached the conclusion that if antiseptic properties are possessed by any of the ingredients used in betel chewing, such properties are not manifested in a demonstrable way in the mouths of chewers or in the expressed juice of the substances employed. This is in complete accordance with the clinical findings of frequent pyorrhea, which theoretically should not occur if the substances chewed were inimical to the growth of bacteria. Betelnut chewing causes retraction and separation of the gums from the teeth and the deposit of concretions. The first certainly and the second probably furnishes physical conditions favorable to periodontal infection, there appears to be no actual antiseptic nor bactericidal action of the substances to prevent such infection occurring.

E RELATION OF BETELNUT CHEWING TO CANCER OF THE MOUTH

That this habit causes chronic irritation of the lips and mouth which predisposes to malignant epithelial growth is a quite common belief, though some writers are indecisive in their statements Coplin⁸ says "Persistent or long continued irritation seems to favor the development of tumors belonging to the epithelial group", as one example of this is cited epithelioma due to chewing betelnut Ewing,⁹ in discussing this question, says "Nevertheless, it is clear, as in Kangri-oven and betelnut cancer of African natives, that local and general predisposition may be ignored, since the tumor develops wherever the irritant is effectively applied" Castellani and Chalmers, in speaking of the betelnut chewing habit in the East, take a rather neutral ground by stating that "the irritation may be the cause of the commonest cancer of old people in these parts" Scheube¹⁰ comments thus "The frequency of cancer of the mouth (lips, mucosa of cheeks, tongue, jaw) in British India is referred to the chronic irritation of betelnut chewing"

During my seventeen months in Siam, I have studied microscopically three specimens of cancer of the lip sent from the operating room of Siriraj Hospital All were from betelnut chewers, although one, a Chinese man, is recorded as chewing betelnut moderately and smoking Siamese cigarets almost constantly, this case, therefore, might seem to be partly a "smoker's" cancer as well as a "betelnut chewer's" cancer In this case, portions of both lips were removed, the lower presenting a warty elevation 2.5 cm long, the upper a grayish, smooth, slightly elevated area The growth on the lower lip proved to be largely a papilloma with early carcinomatous infiltration at the base, the upper lip presented simply thickening of the epithelium with hyperkeratosis A second specimen was a warty growth, 2 by 1.2 cm, on the lower lip of a siamese woman, aged 47 years This also was a papilloma with at points a beginning squamous cell epithelioma The third specimen was from a Siamese man, aged 54 years, who had a rough, slightly elevated growth on the lower lip,—microscopically a papilloma with early carcinomatous extension

It will be noted, that all three specimens were advanced or distinct papillomas, with only early, but also distinct, carcinomatous transformation, the so-called papillary cancer This type of growth appears to be quite frequent in this country in connection with the skin and the mucocutaneous borders That is, there is an extensive papillary growth preceding actual malignant extension, although presumably the latter

8 Manual of Pathology, Ed 5, p 306

9 Neoplastic Diseases, p 460

10 Die Krankheiten der Warmen Lander, p 842

finally occurs in all of them. From the clinical standpoint, surgical removal, even when the growth is large, should result in a large percentage of cures because of the slight infiltration by the tumor.

In addition to these three operative cases, two persons with cancer of the mouth have within the past few months presented themselves at the hospital outpatient department. One of these I saw. The patient was a man, aged 61 years, who had chewed betelnut since he was a boy holding the mass in the left cheek. His teeth had loosened and all fallen out before he was 30 years old. The tumor was an extensive one, involving the left cheek and jaw, and presenting on the external surface as a mass 3 cm in diameter. The other case was one of an ulcerated tumor of the cheek and jaw in an elderly woman. It will be noted that two of these five subjects were women.

Regarding the frequency of tumors elsewhere in the body, the following statement can be made. During the period in which the above three specimens of cancer of the mouth were removed, there were sent from the hospital operating room thirty-two other tumors, of which twenty were malignant and twelve benign. The postmortem service for the same period yielded eleven tumors, eight malignant and three benign.

Through the courtesy of the director, Colonel Phra Sakda, I am enabled to give the statistics of cancer in the Chulalongkorn Hospital of Bangkok for the past six years. They are as follows:

Total number of cases of cancer operated on in hospital, 102
 Sex: Men, 55, or 54 per cent; women, 47, or 46 per cent
 Cancer of lower lip, 52 cases
 Cancer of tongue, 2 cases
 Cancer elsewhere in body, 48 cases
 Sex, cancer of lip: men, 29, or 56 per cent; women, 23, or 44 per cent
 Sex, cancer of tongue: men, 1, women, 1
 Sex, cancer elsewhere in body: men, 25, or 52 per cent; women, 23, or 48 per cent

From this it will be seen that cases of cancer of the lip and tongue comprised more than one-half the total number, an unusual proportion. Virtually all the patients, men and women, had chewed betelnut for many years, the average age being 48 years. Colonel Phra Sakda regards as very significant the fact that in all cases of cancer of the lip, the lower lip was involved, this being the one most injured by betelnut. There are also very few cases of cancer of the tongue, the organ often affected in smokers in other countries.

To me a most suggestive point is that of sex. Statistics from countries in which the people do not chew betelnut show rather uniformly that at least 90 per cent of cancers of the lip are in men. In this series of cases of cancer in betelnut chewers, 56 per cent were

in men and 44 per cent in women, a very striking difference. Of course, this does not prove that the 34 per cent increase above the average for women was due to betelnut chewing. But to what other factor shall it be ascribed? These statistics appear fully to justify the opinion of Colonel Phra Sakda that betelnut chewing is a predisposing cause of cancer of the lip.

As to the frequency of cancer of the mouth in betelnut chewers throughout Siam, the accompanying table summarizes the findings and opinions of a number of physicians in various parts of the country. Most of the figures given are admittedly from memory instead of actual records, and are to be taken in their general significance rather than as accurate statistics.

FREQUENCY OF CANCER OF MOUTH IN BETELNUT CHEWERS IN SIAM

Physician	Years of Observation	Cancer of Lip	Cancer of Cheek	Cancer of Tongue	Cancer of Mouth in Nonchewers	Does Betel Chewing Tend to Cause Cancer
1 E C C	12	5	5	1	None	No
2 C H C	16	None	None	None	None	No
3 W H B	6	None	None	None	None	No
4 E W	35	2	None	None	None	No
5 L C B	4	3	1	1	None	No
6 E B M	18	25	50	None	None	Yes
7 M E B	3	1	None	None	None	No
8 T H H	34	None	None	None	None	No
9 A T B	6	6	1	None	None	No
10 R W M	3	4	6	None	None	No
11 J W M	30	Probably a dozen			None	No
12 G B M	21	About fifteen			None	Yes
13 T D	10	About half a dozen			None	No
14 W B T	28	About half a dozen			None	No
15 C C H	20	About half a dozen			None	No
16 P S	6	Chulalongkorn Hospital			None	Yes

The question answered in the table was "Have your observations led you to believe that betelnut chewing is irritating and tends toward the production of cancer of the mouth?" As thirteen of the sixteen answered this question in the negative, a large majority (81 per cent) is opposed to the view that betelnut chewing causes cancer. But the opinion of one of the three constituting the minority is based on the Chulalongkorn Hospital statistics, the most detailed and significant of them all. Another of the minority is a dentist as well as a physician, and he has specially noted the changes in the mouths of chewers. The third reports many more cases of cancer than the combined numbers of the thirteen joining in the majority opinion.

In the light of the foregoing, what statement is to be made regarding betelnut chewing as a cause of cancer in the people of Siam? One thing is plain, namely, that cancer of the mouth in betel chewers is not frequent enough to have brought it definitely as such to the notice of the majority of the medical profession throughout the country. Therefore, if there is the relation of cause and effect between the two,

it is not apparent to casual observation but must be demonstrated by a special study of the problem and the gathering of statistics. Does the information presented in this paper furnish such demonstration? I think it does not—because it is insufficient in extent. On the other hand, in view of the lack of accurate records by so many of the physicians, and the very significant statistics of the Chulalongkorn Hospital, it surely cannot be said that the opposite has been demonstrated. I believe that the burden of proof is in this instance on the majority. Whether or not this be a just view, the question cannot be answered finally until there has been, for many years, accurate observation and recording of all cases of cancer of the mouth (and of all other parts of the body) throughout the Kingdom.

SUMMARY AND CONCLUSIONS

1 Betelnut chewing is a well-nigh universal habit of the Siamese people

2 Decay of the teeth is prevented almost completely, apparently by the deposit of concretions

3 Neither antiseptic nor bactericidal action on the flora of the mouth is exerted by the substances chewed

4 Direct constitutional effects of the habit appear to be negligible

5 Betelnut chewing leads to chronic changes in the mucous membrane of the mouth, recession of the gums, pyorrhea alveolaris, deposit of lime concretions on the teeth, atrophy of alveolar processes and loosening and loss of teeth

6 The exact way (or ways) in which teeth are loosened is not well understood

7 As the loss of teeth appears to be the most serious result of betelnut chewing, a thorough study of its mechanism and prevention is eminently desirable

8 Betelnut chewing does not frequently cause cancer of the mouth, but there are reasons for believing that, in an as yet undetermined percentage of cases, it does lead to this result

CAROTINEMIA

REPORT OF A CASE IN AN ADULT

GEORGE DOUGLAS HEAD, M.D., AND REUBEN A JOHNSON, M.D.
MINNEAPOLIS

Under the term carotinemia, Hess and Myers¹ described the occurrence of a peculiar yellow discoloration of the skin of the body with the presence of carotin, the characteristic pigment of carrots, in the blood of children fed on a diet containing carrots. We wish to report a similar occurrence in an adult, a case in which we were able to establish the casual relationship between diet and pigmentation, and to identify the offending pigment chemically, in a relatively large amount, in the blood.

The yellow pigment carotin is not confined to carrots. It is included with xanthophylls under the general term carotinoids. They are widely distributed in nature, often in close association, and can be separated from each other on the basis of solubility. The yellow color of milk fats, corpus luteum and egg yolk, is due to carotinoids which are sometimes called lipochromes and again, lutein. Among human vegetable food stuffs they are found especially in spinach, lettuce, oranges and carrots. Palmer² and his associates have done splendid work in clearing up the problems of carotinoid pigmentation. They have shown conclusively that the natural pigment of egg yolk, body fat and blood serum of the hen is physiologically identical with the carotin and xanthophylls of plants, with the latter present in far greater proportion. In the cow, the coloring of milk fat, body fat, skin secretions, corpus luteum and blood serum is similarly due to carotinoids, but in contrast to the hen, the carotin is in excess with only traces of xanthophylls. They were able to produce almost colorless milk fat or egg yolks by feeding a diet poor in the respective characteristic pigment. Fat is the storehouse of carotinoids and the absence of these pigments explains the colorless fat of swine, sheep and goats. There is, therefore, a wide variation among species in their behavior toward these pigments. Analysis of the fat of human milk showed tinting with both carotin and xanthophylls in nearly equal proportions. They concluded that the pigment of human fat is, no doubt, identical with the pigment of human milk fat.

With the exception of the article of Hess and Myers and an editorial³ reference, we have found no description in the English litera-

1 Hess, A. F., and Myers, V. C. Carotinemia. A New Clinical Picture, *J. A. M. A.* **73** 1743 (Dec. 6) 1919.

2 Palmer, L. S., and Eckles, C. H. *J. Biol. Chem.* **17** 191, 211, 223, 237, 245 (March) 1914, Palmer, L. S. *Ibid.* **23** 261 (Nov.) 1915, **27** 27 (Oct.) 1916.

3 *J. A. M. A.* **74** 32 (Jan. 3) 1920.

ture of a case presenting pigmentation resulting from a diet rich in carotin. A number of articles have appeared in the German literature, especially by pediatricians, during the period of the war when food shortage necessitated a change to a more vegetarian diet. Kaupe⁴ observed, in a childrens home, a large number of otherwise healthy children, who showed a marked yellow pigmentation of the skin of the face. Carrots were present in the diet of all those affected but not all the children who ate carrots showed the discoloration. When carrots were withdrawn from the diet, the color disappeared and could be brought back by adding carrots to the diet again. The observations of Kaupe were confirmed by Stoelzner⁵ who had seen many similar cases which he called pseudo-icterus. Klose⁶ also reported cases of carrot pigmentation, and thought that well nourished children were more prone to the affection, probably dependent on the affinity of the fats for carotinoids. Many years ago, following the use of carrot soup, Moro⁷ had seen a yellow discoloration of the skin develop. The actual presence of carotin in the blood was not established by chemical tests in any of the above reported cases.

The condition is not confined to the early years of life. Umber⁸ used the term "Diabetische Xanthose" to describe cases of marked yellow skin pigmentation which he had seen in diabetics. He had seen fifteen cases in all and thought that the color varied with the intensity of the disease. The blood, however, contained an ochre gold color, not due to bile, but extractable with ether. The term "Xanthosis Diabetica" was originally used by von Noorden⁹ to describe similar cases in diabetics of skin pigmentation varying from a canary yellow to a deep orange gold. In commenting on the reports by pediatricians of pseudo-icterus following the feeding of carrots, Saloman¹⁰ states that it corresponds entirely to the description given by Von Noorden and himself of xanthosis. He had seen many cases, usually in diabetics, but several times in entirely normal people. Spectroscopic examination of the blood serum showed absorption bands identical with those of carotin. Schuessler¹¹ observed a marked golden

4 Kaupe, W. Hautverfärbung bei Säuglingen und Kleinkindern infolge der Nahrung, München med Wchnschr 66 330 (March 21) 1919

5 Stoelzner, W. Ueber Pseudoikterus Nach Mohrrubengenuss, München med Wchnschr 66 419 (April 11) 1919

6 Klose, E. Hautverfärbung bei Säuglingen und Kleinkindern infolge der Nahrung, München med Wchnschr 66 419 (April 11) 1919

7 Moro, E. München med Wchnschr, 1908, p 1562, *ibid* 66 674 (June 13) 1919

8 Umber. Diabetische Xanthose, Berl klin Wchnschr 53 879 (July 24) 1916

9 Von Noorden. Internat Dermatologenkongress 1904

10 Saloman, H. Von Noorden's Handbuch der Pathologie des Stoffwechsels, Ed 2 2 290, München med Wchnschr, May 23, 1919, p 564

11 Schuessler. Ueber Hautverfärbung durch Mohrrubengenuss, München med Wchnschr 66 596 (May 30) 1919

brown (orange) pigmentation of the entire body, without involvement of the sclerae, in three men past middle life who had eaten heavily of carrots as a substitute for potatoes, which were scarce. He made no chemical analyses, and his only explanation for the phenomenon was the excessive ingestion of carrots.

In studies of the pigments found in human blood serum, Hymans van den Bergh and Snapper¹² found serums showing a high lipochrome content. In some of these cases, usually diabetics, a peculiar orange yellow color of the skin, without scleral involvement, was present. Buerger and Reinhart¹³ studied a number of cases of xanthosis. They found that there was an increased quantity of lipochrome in the blood serum, that the xanthosis and the parallel increase of lipochrome in the blood came from the food and could be influenced by diet, and that the lipochrome corresponded in chemical and spectroscopic tests to the carotinoid group of pigments which are closely associated with chlorophyll in green plants. Hymans van den Bergh and Mueller¹⁴ confirmed the observation of the dependence of blood lipochrome on the lipochrome content of food. They were able to increase the normally small and somewhat variable quantity of lipochrome in human serum by feeding a diet rich in green leafy vegetables and eggs, and they found increased values in the summer months when vegetables of this type are eaten more freely. The diet peculiar to diabetics leads to an increase of pigment in the blood, and, therefore, to the development of xanthosis. Carotin was found in excess of xanthophylls. They also estimated the lipochrome content of various organs in many diseased states and found a surprisingly high percentage in the suprarenals with lesser quantities in the liver, spleen, fat and blood in the order given. The lipochrome content of the organs seemed independent of the amount in the blood or of any particular disease.

The condition is apparently harmless as no symptoms are ascribed to it. Furthermore, Wells and Hedenburg¹⁵ found no toxic effects in guinea-pigs from intraperitoneal injection and none of consequence on intradermal injection of carotin extracted from carrots, dissolved in olive oil, and used in relatively enormous doses. It is probable, that the carotinoids perform no useful physiologic function in the body.

12 Hymans van den Bergh and Snapper. Die Farbstoff des Blutserums, *Deutsch Arch f klin Med* **110** 540, 1913.

13 Burger, M., and Reinhart, A. Ueber die Genese der Xanthose diabetica, *Deutsch med Wchnschr* **45** 430 (April 17) 1919, *Ztschr f d ges exper M* **7** H 3.

14 Hymans van den Bergh and Muller, P. Das lipochrome Pigment in Blutserum und Organen, Xanthosis, Hyperlipochromamie, *Biochem Ztschr* **108** 279 (May) 1920.

15 Wells, H. G., and Hedenburg O. F. The Toxicity of Carotin, *J Biol Chem* **27** 213 (Oct) 1916.

An abnormal pigmentation of the skin, therefore, has been seen to arise from a diet containing unusual amounts of carotinoids. The color is described as varying from a lemon yellow to a rich orange in well developed cases, and can easily be differentiated from jaundice by the difference in color and the fact that the sclerae are not affected. Some portions of the body may be more heavily tinted than others, the palms of the hands, the soles of the feet, the forearms, sternum, the nasolabial folds, cheeks and forehead are especially mentioned in some reports. There is apparently some variation in individuals with respect to the ease with which the pigmentation may develop, possibly being more likely to occur in well nourished persons. When the excess of carotin bearing food is removed from the diet, the skin resumes its normal color more or less slowly. It is reasonably certain that the xanthosis diabetica of the older writers is carotinemia without a correct interpretation having been made. Several observers had previously suspected, from spectroscopic study, the true nature of the serum pigments, but the demonstration of an increase of the carotinoid group of pigments in the blood serum, dependent solely on the same pigments in the food supply in these cases of pigmentation, is a very recent achievement. The condition produces no harmful effects.

REPORT OF CASE

History—J. B., male, aged 35, bank clerk. Family and previous history unimportant. He was first seen in January, 1919, presenting the classical symptoms of diabetes mellitus which had been present in severe form for about two weeks. He was then on a restricted carbohydrate diet prescribed by his local physician. Physical examination showed nothing of importance aside from moderate emaciation. The urine contained a moderate amount of sugar and acetone bodies. Hemoglobin, 78 per cent. Starvation for twenty-four hours cleared up the glycosuria following which a fairly liberal diet was gradually established without its reappearance. He was taught how to test his urine for sugar and was sent home with instructions to report at intervals for examination.

The next visit was in April, 1919, at which time he called attention to a light yellow tinging of the skin which he and others had noticed. A correct explanation could not be given but a true jaundice was ruled out on the absence of symptoms, bile in the urine and discoloration of the sclerae. Examination of the eye grounds by Dr. Campbell of Minneapolis, showed no fundus changes, and the visual disturbance of which the patient complained was explained as astigmatism. In August, 1919, he was still sugar-free, the Wassermann was negative, and the blood showed a moderate secondary anemia. In November, 1919, he returned feeling well, with a fairly high carbohydrate tolerance and a blood sugar reading of 0.148 per cent. In February, 1920 sugar appeared in the urine with the blood sugar 0.18 per cent, after a fairly large carbohydrate intake.

He had not been seen for about a year when he reported for examination in January of this year. During the entire period since he first presented himself many urinalyses by himself (almost daily) and our laboratory, showed no sugar, except when determining tolerance. Acetone bodies had been consistently absent. He had held his weight and strength and his general condition was excellent. In his correspondence he referred from time to time to the yellow color which he had already called attention to in one of his early visits.

Examination—His appearance was certainly very striking. There was a high grade, diffuse, generalized, bright orange yellow pigmentation of the skin of the entire body, varying somewhat in its intensity of distribution, being especially vivid in the palms of the hands. The sclerae were normal. There were some dirty brown chloasma-like patches on the skin of the upper abdomen. He stated that the yellow color would last only a few days, sometimes three, his wife might also notice the heightened color and then it would suddenly disappear. This seems to us to be faulty observation as it had been present for many months and did not behave in this manner while under direct observation. He had no abdominal pain and no discomfort whatever but worried constantly about his unusual appearance as he was fearful that it meant some serious derangement. The blood sugar was 0.167 per cent. The urine was free from sugar, acetone, diacetic acid and bile pigments. Blood examination showed hemoglobin, 73 per cent, erythrocytes, 4,475,000, leukocytes, 5,250. Differential count: polymorphonuclears, 57.5 per cent, lymphocytes, 33 per cent, large mononuclears, 5 per cent, transitionals, 2 per cent, eosinophils, 2 per cent, basophils, 0.5 per cent. Inquiry showed that he had eaten heavily of cabbage and carrots and scarcely ever ate any other vegetables.

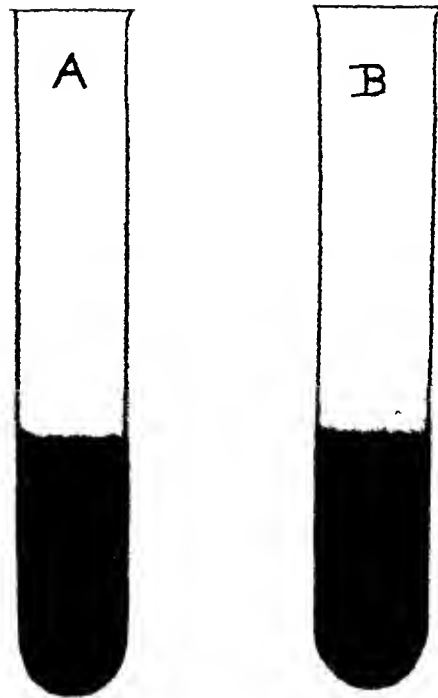
A diagnosis of carotinemia was made and carrots were eliminated from his diet immediately. He was kept under observation for a period of six days during which a perceptible fading of the color occurred. About 20 c.c. of blood were drawn by venipuncture. A small amount of the blood was allowed to coagulate in a test tube, and the supernatant serum showed a bright golden yellow color (Fig 1) which contrasted strongly with the straw color of normal serum (Fig 2).

The remainder of the blood was oxalated and sent to Leroy S. Palmer, associate professor of agricultural biochemistry of the University of Minnesota, whose brilliant researches with carotin have been mentioned previously. His report reads:

I have examined the sample of blood from the case of suspected carotinemia and beg to report as follows. There is no question regarding the presence of carotin in relatively large amounts in this blood, the serum color being due almost exclusively to this pigment. It is likely that traces of xanthophylls, the other class of carotinoids, are present also. This being the first sample of human blood I have had an opportunity to examine for carotinoid pigments I took occasion to obtain an approximate estimation of the amount of pigment present. So far as I am aware, no measurement of carotinoids in human blood has heretofore been made, although a procedure for the determination of these pigments in other materials has been worked out. The result of the quantitative determination showed approximately 0.00057 gm. carotin per 100 c.c. of blood. This is, of course, a very small amount. However, carotin is a very intense pigment. Just how intense it is may be judged from the fact that butterfat from cows on fresh pasture grass contains on the average 0.025 gm. carotin per kilogram of fat. Assuming 5 per cent blood in the human body, an individual weighing 75 kg. with 0.00057 gm. carotin per 100 c.c. of blood would have sufficient carotin in the entire blood to color about 1 kg. of "June" butterfat. I thought these figures might interest you.

Permit me to say a word regarding the application of this examination to the case in question. It is only fair to point out that the finding of carotin in this blood is not necessarily a sure criterion of the carotinemia suspected. Carotin is unquestionably a normal constituent of human blood, the amount depending on the character of the diet. Carotinoids have already been reported in human blood from individuals presumably not suffering from carotinemia. I have found the pigments to be normal, although of varying consti-
tution in human milk fat. Human adipose tissue is notoriously yellow, as you know, due to the same pigments. The only sure index of the carotinemia suspected will be the disappearance of the pigment from the skin, at least so it seems to me now that the source of the pigment has been removed. This will be slow, judging from my experience with animals and the cases reported by Hess with children. It might be of interest to examine the blood again.

After being on the carrot-free diet for one month the patient wrote: "The yellow is very nearly gone, the face and the rest of the body is clear. The palms of the hands show a trace of it yet. I am getting along fine." After two months he wrote: "The yellow color is very nearly gone but there is a trace of it left yet on the inside of my fingers."



A orange yellow color of serum of patient | B straw color of normal serum for comparison

SUMMARY

We report a case of marked orange yellow skin pigmentation, most intense in the palms of the hands, without involvement of the sclerae, in an adult with moderately severe diabetes. Carrots had formed a heavy component of the previous diet. The blood serum showed a bright golden yellow color which was chemically demonstrated to be due to carotin. The urine showed no bile pigments, and withdrawal of the carrots from the diet caused a disappearance of the pigmentation.

FOCAL INFECTION AND ELECTIVE LOCALIZATION IN THE ETIOLOGY OF MYOSITIS*

EDWARD C ROSENOW, M D, AND WINIFRED ASHBY, PH D
ROCHESTER, MINN

Although acute muscular rheumatism is generally conceded to be an infectious process, the myalgias and milder forms of myositis are not so definitely accepted as such. Myalgic pains are considered by Taylor¹ to be the result of faulty metabolism, by Gordon² of excessive katabolism from over-exertion of muscle cells, and by Profanter³ as the peripheral expression of pain caused by defective function or injury of visceral organs. Except among English writers, such as Poynton,⁴ Llewellyn and Jones,⁵ Munroe and Almond,⁶ and Stockman and Renton,⁷ little credence is given to the idea of localized invasion of the muscles by bacteria other than in marked acute generalized myositis, and the condition described as dermatomyositis.

The demonstration by one of us (Rosenow⁸) eight years ago that streptococci from patients with rheumatic fever with muscular involvement, and streptococci from patients with acute nonrheumatic myositis,⁹ tended to localize and produce nonsuppurative lesions in muscles of animals following intravenous injection supports strongly the infectious theory of myositis. The localization in muscles occurred with strains isolated from the focus of infection as well as those from the involved muscles.

There are many reasons for believing that chronic localized infections, such as in tonsils and teeth, play an important part in the production of pronounced and mild forms of myositis. Infections in these areas are commonly present in patients suffering from myositis. Localized myositis occurs often during certain epidemics of tonsillitis and other infections of the upper respiratory tract. Rheumatic fever in

*From the Division of Experimental Bacteriology, The Mayo Foundation

1 Taylor, J M. On Myalgic Pains, *Lancet* **2** 976, 1919

2 Gordon, A. Myalgia of an Isolated Portion of a Muscle, *New York M J*, **107** 113, 1918

3 Profanter, P. Das Problem des Muskelrheumatismus, *Ztschr f diatet u physik Therap* **23** 121, 1919

4 Poynton, F J. A Contribution to the Study of Acute Rheumatism, *Brit M J*, **1** 371, 1919

5 Llewellyn, L J and Jones, A B. *Fibrositis*, New York, Rebman Co, (n d), pp 693

6 Munroe and Almond. Quoted by Llewellyn and Jones

7 Stockman and Renton. Quoted by Llewellyn and Jones

8 Rosenow, E C. The Etiology of Articular and Muscular Rheumatism *J A M A* **60** 1223 (April 19) 1913

9 Rosenow, E C. Elective Localization of the Streptococcus From a Case of Pulpitis, Dental Neuritis and Myositis, *J Immunol* **1** 363, 1916

which involvement of muscles is common is usually ushered in by an attack of tonsillitis. Beneficial effects from tonsillectomy were noted by Lillie and Lyons¹⁰ in a study of 200 cases of myositis and arthritis, by Nordlund¹¹ in thirty cases, and by Kelly¹² in ten cases. The nature of the causal relationship, however, is generally considered to be other than the localization of bacteria in the muscles. Krebs¹³ and Schmidt¹⁴ put forward the idea that the symptoms in these conditions are due to injury of the nerves by toxins produced by the bacteria in a focus of infection.

The difficulties in studying the etiology of myositis, especially the milder forms, are many. Attacks of myositis are rarely fatal. Cultures from the blood prove negative. The lesions often occur in inaccessible parts. Removal of pieces of muscle for cultural and other studies is painful and objected to by many patients, and the isolation of bacteria free from contamination is difficult. We endeavored to obtain light on the nature of this puzzling problem by testing the effect in suitable animals of the intravenous injection of the bacteria from foci of infection, and excised pieces of muscles in cases of myositis and closely related conditions, and by noting clinically the effect on the course of the disease of the removal of the suspected foci of infection.

TECHNIC

The technic has been similar to that employed in other elective localization studies.¹⁵ Whenever possible, material obtained from the depths of the suspected focus of infection, that is, material expressed from tonsils or aspirated from the depths of pus pockets of teeth showing pyorrhea, and the washings from ground-up tonsil tissue, was injected intravenously immediately. Cultures were made in tall columns (from 10 to 12 cm.) of ascites-glucose broth or glucose-brain broth (0.2 per cent), and on blood-agar plates. To each tube of broth adjusted to hydrogen ion of 6.7 was added approximately 2 cc. of calves' brain before autoclaving. The animals were injected intravenously with either the young primary broth culture or with the centrifuged organisms suspended in salt solution. From 2 to 5 cc. of the broth culture itself, or the growth from 10 to 30 cc. in salt solution were used routinely in average sized rabbits. The dose for guinea-pigs and dogs was in proportion. Smears and blood-agar plates were made of the material injected to determine the character of the organisms and their viability. Cultures made

10 Lillie, H. I., and Lyons, H. R. Tonsillectomy in Myositis and Arthritis, *J. A. M. A.* **72** 1214 (April 26) 1919.

11 Nordlund, H. Outcome after Tonsillectomy for Rheumatism, *Hygiea*, **81** 497, 1919, Abstr. *J. A. M. A.* **73** 568, 1919.

12 Kelly, T. H. The Result of Animal Inoculations with Material Obtained From the Tonsils of Cases of Acute Rheumatic Fever, *Ohio M. J.* **14** 221, 1918.

13 Krebs, W. Beitrag zur Frage der Myalgie und ihrer Behandlung, *Ztschr. f. diätet. u. physik. Therap.* **23** 288, 1919.

14 Schmidt, A. Zur Pathologie und Therapie des Muskelrheumatismus (Myalgie), *München med. Wchnschr.* **1** 593, 1916.

15 Rosenow, E. C. Elective Localization of Streptococci, *J. A. M. A.* **65** 1687 (Nov. 13) 1915, Studies on Elective Localization. Focal Infection with Special Reference to Oral Sepsis, *J. Dent. Res.* **1** 205, 1919.

from plated material were not used routinely, for it has been shown that the bacteria having elective localizing power may be sensitive to oxygen, especially in the primary culture, and often fail to grow on blood-agar plates, and when not, aerobic cultivation has been found to destroy the peculiar pathogenicity which enables them to invade specific tissues. In shake cultures of excised pieces of muscle, in tall columns of agar the organisms may retain their viability and specific localizing power for a long time (Case 130).

Usually only one injection was given, in some instances two were given, and rarely three. The animals were often caged separately, observed for evidence of muscle symptoms, and handled with care to avoid injury. Those that died were examined as soon after death as possible. Those that survived were etherized, as a rule in from forty-eight to seventy-two hours after the last injection and examined at once. In special instances they were allowed to live for from ten to fourteen days. Painstaking search for focal lesions in muscles and viscera was made in a strong light, and the incidence of lesions recorded.

The identity of the organism causing the lesions was determined by making routine cultures on blood agar and in tall columns of the broth of emulsion of the lesions in the muscles, of the blood (0.2 c.c.), and, in some instances, of emulsions of normal muscle, and viscera showing no lesions, and by microscopic examination of stained sections of the tissues. The organism isolated from the lesions in the muscles was frequently reinjected one or more times.

RESULTS

In this way we studied altogether twenty-eight cases of myositis, extending over a period of six years. The important clinical and experimental findings in these cases are summarized in Tables 1, 2 and 3. The age of the patients ranged from 6 to 65 years. All patients, except one, were 20 or more years of age. Sixteen were males and twelve were females. The condition found in the patients varied from mild acute transient, often recurring, attacks of localized myositis in persons who were well otherwise, to marked chronic generalized myositis which had incapacitated them for work for several years. Some of the patients had had attacks of other diseases, such as cholecystitis, but these conditions were not manifest at the time of our study. In six cases one or more other members of the family were similarly afflicted. There was a history of tonsillitis or sore throat in relation to attacks or exacerbations in ten cases. The tonsils were usually small and appeared to be quite normal on inspection, but by means of pressure pus was often expressed, especially from the pole, indicating probable absorption of bacteria and their products.

The tonsils alone were removed as the focus of infection suspected of having causal relationship in thirteen cases, teeth alone in four cases, and infected teeth and tonsils in eight cases. In three cases no foci were removed. In these cases the muscle trouble was preceded by tonsillitis in two and by nasopharyngitis in one.

The extirpated tonsils often showed one or more abscesses near the base. The infections of the teeth included pyorrhea (Case 601) infection of the pulp without apical rarefaction (Case 567), and apical

TABLE 1.—SUMMARY OF CLINICAL AND EXPERIMENTAL FINDINGS IN CASES OF UNCOMPLICATED MYOSITIS

Case	Age, Sex	Clinical Findings	Foci Removed	Result	Bacteria Having Elective Localizing Power	Animal Experiments													
						Animals Infected	Joint Fluid (turbid)	Periarticular Ligaments	Muscles	Nerves	Stomach and Duodenum	Kidneys	Lungs	Thyroid	Skin	Pericardium	Myocardium	Endocardium	Other Organs
130	21 F	Tonsillitis and puerperal fever two years before, followed by tender, painful nodes in the muscles of the neck and arms, and thyroid gland, excised nodes from neck showed encysting myositis	None		Slightly hemolyzing streptococcus from muscle	38	4	1	29	0	6	3	1	6	3	0	10	0	Gallbladder, suppurative, appendix, pancreas, and liver in one each, intestines, thymus lymph gland and eyes in two each
593	36 F	Acute pain in localized areas in muscles of back following tonsillitis, spontaneous recovery	None		Undetermined	1	0	0	1	0	0	0	0	0	0	0	0	1	
594	6 M	Acute pain in muscles of shoulder and neck following nasopharyngitis, spontaneous recovery	None		Undetermined	1	0	0	1	0	0	1	0	0	0	0	0	0	
602	26 M	Cervical myositis following tonsillitis	Tonsils	Unknown	Slightly hemolyzing streptococcus and streptococcus from tonsils	1	1	0	4	0	1	2	0	0	0	0	3	0	Testicle in one
623	36 M	Myositis of right gluteus minimus of four years' duration, marked improvement since tonsillectomy one year before, tender tooth found and removed, encysting pulpitis	Infected tooth	Complete recovery	Slightly hemolyzing streptococcus from calcifying dental pulp	2	1	0	2	0	0	0	0	0	0	0	0	0	
624	48 M	Chronic lumbar myositis for the last eight years following attacks of tonsillitis, septic tonsils, in bed for eight weeks with sciatica one year before	Tonsils	Complete recovery during last two years	Green producing streptococcus from tonsils	2	0	0	2	0	0	0	0	0	0	0	0	0	
901	37 M	Marked chronic generalized myositis which began two years before with severe pain in muscles of back and legs after exposure to wet and cold, muscles and joints stiff and cold, eosinophilia of 47 per cent, leukocytes 5,000, urine normal, tonsils infected	Tonsils	Gradual but complete recovery from myositis	Slightly hemolyzing streptococcus from tonsils	7	2	1	6	1	0	2	0	0	0	1	1	1	Brain in one, liver in two
909	26 F	Recurring attacks of tonsillitis followed by cervical myositis, the attack studied came on ten days after acute follicular hemolytic streptococcal tonsillitis associated with fever and moderate leukocytosis, abscesses in calcified tonsils	Tonsils	Recovery	Green producing and indifferent streptococcus from tonsils	11	3	2	11	0	0	1	2	0	0	0	2	0	
912	30 F	Mild recurring attacks of localized myositis, and cholecystitis, two attacks of cholecystitis, one associated with pericarditis, one infected tooth, and in infected tonsils	Tonsils and one infected tooth	Improvement	Slightly hemolyzing and green producing streptococcus from tonsils and tooth	12	1	1	12	0	1	0	0	0	0	0	0	0	
1035	20 F	Mild recurring attacks of localized myositis, and probably cholecystitis, attacks usually followed tonsillitis, tonsils showed a number of abscesses	Tonsils	Improvement	Slightly hemolyzing streptococcus from tonsils	12	2	0	11	0	0	1	0	0	0	0	0	0	
Total (10 cases)						90	14	5	79	1	8	10	3	6	1	1	16	2	

TABLE 2—SUMMARY OF CLINICAL AND EXPERIMENTAL FINDINGS IN CASES OF MYOSITIS AND ARTHRITIS

Case	Age, Sex	Clinical Findings	Foci Removed	Result	Bacteria Having Elective Localizing Power	Animal Experiments														
						Number Showing Lesions in														
						Animals In jected	Joint Fluid (turbid)	Periarticular Ligaments	Muscles	Nerves	Stomach and Duodenum	Kidneys	Lungs	Thyroid	Skin	Pericardium	Myocardium	Endocardium	Other Organs	
104	10 M	Recurring localized myositis, subacromial bursitis, typical painful shoulder, many small abscesses in tonsils, infected devitalized teeth, painful bleeding hemorrhoids, with superficial ulceration	Tonsils and two infected teeth	Marked improvement	Slightly hemolyzing streptococcus from tonsils and teeth	8	2	1	5	0	3	0	0	2	0	0	1	2	Gallbladder in one	
309	25 M	Chronic localized myositis, chiefly of muscle insertions around joints, and chronic arthritis	Tonsils, tonsil tag, and infected tooth	Improvement after tonsillectomy, complete recovery after removal of tonsillar tag and tooth	Green producing streptococcus from tonsils, tooth, and joint fluid	8	4	2	4	0	3	1	0	0	2	3	3	2		
536	27 M	Muscular and articular rheumatism for three years, repeated attacks of tonsillitis	Tonsils	Decided improvement, but not complete recovery	Staphylococcus aureus from tonsils	4	2	1	4	0	1	2	0	0	0	0	3	1	Bladder in one	
542	42 F	Repeated attacks of rheumatic fever preceded by attacks of sore throat, chronic myositis and arthritis, chiefly of knees, elbows, shoulders, and back, hypertrophic rhinitis, follicular pharyngitis, large septic tonsils two abscessed teeth	Tonsils and two abscessed teeth	Gradual improvement after tonsillectomy, rapid improvement after removal of abscessed teeth	Slightly hemolyzing streptococcus from tonsils and teeth	1	0	1	1	0	0	0	0	0	0	0	0	0		

		Chronic myositis and peri-arthritis, septic tonsils	Tonsils	Marked improvement	Slightly hemolyzing streptococcus from tonsils	2	1	1	2	0	0	0	0	0	0	0	1	2	Gallbladder in one
543	65 M																		
552	51 M	Rheumatic fever when a child, repeated attacks of muscular and neuralgic pains, lumbago and sciatica for twelve years, repeated sore throat, pericarditis three years before, myositis and arthritis of right knee but no sciatica at time of examination, abscesses in tonsils	Tonsils	Complete recovery soon after removal of tonsils five years before	Green producing streptococcus from tonsils	2	1	0	2	0	1	0	0	0	0	0	1		
621	53 F	Chronic localized myositis, peri-arthritis and arthritis, tonsillectomy seven years before, with temporary improvement infected tonsil tissue at base of tonsillectomy scar, dental abscesses	Tonsil tag, and two infected teeth	Decided improvement, but not complete recovery	Green producing streptococcus from infected teeth	4	3	3	2	0	1	0	0	0	0	0	0	0	Appendix, and hyperemia of pulps of teeth, one each
622	42 M	Chronic recurring localized myositis subacromial bursitis, and low grade peripheral arthritis of fingers, tonsillitis, infected tonsillar tissue following incomplete tonsillectomy, dental infection	Tonsils and in devitalized teeth	Repeated improvement of systemic condition following removal of foci	Slightly hemolyzing and green producing streptococcus from tonsils and teeth	17	3	5	12	0	0	1	2	0	0	0	1	0	Bursae in nine, bladder in one
898	34 F	Chronic arthritis and myositis, marked exacerbation following quinsy three months before tonsils small and rigid, apical infection of two devitalized teeth	Tonsils	Unknown	Green producing streptococcus from tonsils	1	0	0	1	0	0	0	0	0	0	0	1	0	
913	37 F	Chronic localized myositis, peri-arthritis and bursitis, submerged, small, septic tonsils	Tonsils	Complete recovery	Slightly hemolyzing streptococcus from tonsils	12	1	5	11	0	1	1	1	0	1	1	1	0	Bursae in four lymph glands in two, spleen in one
922	64 F	Chronic myositis and arthritis and paronychia unguitum	One infected tooth	Temporary improvement of joint and muscle symptoms	Green producing streptococcus from tooth	2	0	0	2	0	0	0	0	0	0	0	0	0	
		Total (11 cases)				61	17	19	46	0	10	5	3	2	3	1	12	8	

infections of artificially devitalized teeth (ten cases) Of the twenty-five patients from whom one or more suspected foci were removed, improvement or complete recovery, which was attributable to this treatment, occurred in twenty-two In two cases (Cases 602 and 898)) the result is unknown, while in one case (Case 572) no improvement followed The improvement in many instances occurred promptly, especially in the more acute cases In the more chronic and more extensive infections the improvement was slow and sometimes did not become apparent until several months had elapsed The good effects were not merely temporary, complete recovery has lasted in many of the cases for a number of years In three cases partial recovery, noted after a previous tonsillectomy, became complete, or additional improvement occurred following the removal of the dental infections (Cases 567, 623, and 4132) In two patients complete restoration to health followed removal of tonsillar tissue which remained after a defective tonsillectomy, and the removal of one or more infected teeth (Cases 369 and 622)

In all cases but one, a patient with spasmodic torticollis (Case 572), in whom no improvement followed tonsillectomy, elective localization of bacteria from foci or from excised muscle occurred following their intravenous injection into animals Of the twenty-seven positive cases, the streptococcus was found to have elective affinity for the muscles in twenty-four cases, the streptococcus and staphylococcus in two cases, and the staphylococcus alone in one case

The streptococcus produced two types of colonies on blood-agar plates, one, a small colony surrounded by a narrow and usually a somewhat hazy zone of hemolysis, the other, a slightly larger but dry colony surrounded by a greenish zone The muscle lesions in animals were caused by the slightly hemolyzing streptococcus in ten cases and by the green-producing type in ten cases, in four cases both varieties were present, and in two cases the hemolyzing streptococcus and staphylococcus (Case 602) were responsible In one case the *Staphylococcus aureus* alone (Case 536) was the causative factor.

In most instances, the details of the patients' symptoms were not known to us when the results of the experiments were recorded In reviewing the clinical histories, we found that the cases fell into three quite distinct groups The cases in Group 1 (summarized in Table 1), comprise the cases of acute and chronic myositis without other demonstrable lesions at the time of study In Group 2 (Table 2) are the cases that presented predominating symptoms of myositis, but in which periartthritis and arthritis were also present In Group 3 (Table 3) are the cases in which the findings were chiefly those of myositis, but in which symptoms of mild neuritis or perineuritis were

TABLE 3.—SUMMARY OF CLINICAL AND EXPERIMENTAL FINDINGS IN CASES OF MYOSITIS AND NEURITIS

Case	Age, Sex	Clinical Findings	Toel Removed	Result	Bacteria Having Elective Localizing Power	Animal Experiments														
						Number Showing Lesions in														
						Animals In-jected	Joint Fluid (turbid)	Periarticular Ligaments	Muscles	Nerves	Stomach and Duodenum	Kidneys	Lungs	Thyroid	Skin	Pericardium	Myocardium	Endocardium	Other Organs	
567	28 F	Neurasthenia, migraine, recurring acute myositis of muscles of neck, associated with dental neuritis or facial neuralgia, cervical lymphadenitis, colloid goiter, three infected dental pulps, previous tonsillectomy with improvement	Infected teeth	Marked im- provement, but not complete recovery	Slightly hemolyzing streptococcus from teeth, and excised muscle	23	0	1	15	12	3	3	1	0	0	0	0	0	Teeth in eleven, gallbladder in two, supraren- als, spleen, and lymph glands, one each	
571	22 F	Neurasthenia, migraine, pains in muscles of chest, especially the left side, and in the back, numbness of right arm, espe- cially when fatigued and nervous, hack- ling cough, and nausea, infected tonsils, cloudy right antrum	Tonsils	Marked im- provement	Green produe- ling strepto- coccus from tonsils	5	2	1	5	2	1	1	1	0	0	0	1	1		
572	13 M	Spasmodic torticollis for four months	Tonsils	No change in condition	None	2	0	0	0	0	0	0	0	0	0	1	0	0		
601	13 M	Repeated attacks of muscular rheuma- tism for years at time of examination impaired conduction and heart block marked pyorrhea, apical infection of three teeth	Three teeth, properly incised treat- ment of pyorrhea	Marked im- provement lasting two years	Slightly hemolyzing streptococcus from teeth	13	0	6	7	2	0	0	5	0	0	0	3	5	Spinal cord, aorta, intestines, two each, gall bladder, thymus epieural fat one each	
923	14 M	Neurasthenia, lack of endurance, muscu- lar pains, especially of the chest and shoulders, numbness in arms at times, apical infection around three teeth, septic tonsils	Tonsils, and three infected teeth	Gradual im- provement	Green produe- ling strepto- coccus from tonsils and teeth	4	0	0	3	0	1	0	0	0	0	0	0	0		
2291	19 M	Neurasthenia, lack of endurance, muscu- lar and nerve pains in lumbar region, and thighs, septic tonsils	Tonsils	Gradual im- provement	Staphylococ- cus aureus from tonsils	2	0	0	2	1	0	0	1	0	0	0	0	0		
1112	6 M	Acute localized myositis and dental neu- ritis previous tonsillectomy with im- provement	Three infected teeth	Prompt im- provement	Green produe- ling strepto- coccus from teeth	2	0	0	2	1	0	0	0	0	0	0	0	0	Teeth in two	
Total (7 cases)						51	4	8	34	18	8	1	8	0	1	3	6	7		

also present. The incidence of lesions in each of the three groups is given in Tables 1, 2 and 3. It will be noted that in Group 1, the pure myositis group, seventy-nine (88 per cent) of ninety animals injected had lesions in muscles, fourteen (16 per cent) animals had turbid joint fluid, and only 1 per cent had lesions in nerves. In Group 2, that of myositis and arthritis, lesions of muscles occurred in forty-eight (79 per cent) of the sixty-one animals injected, seventeen (28 per cent) had turbid joint fluid, and none developed lesions in nerves. In Group 3, that of myositis and neuritis, lesions in muscles developed in thirty-four (67 per cent) of fifty-one animals, only four (8 per cent) had turbid joint fluid, and eighteen (35 per cent) had lesions in nerves or nerve sheaths. The incidence of lesions in the periarticular ligaments in the three groups was 6 per cent, 31 per cent and 16 per cent, respectively. This parallels quite closely the patients' symptoms, not only with regard to the chief lesions in the muscles, but also in the minor lesions, such as those in the joints in the arthritis group and in the nerves in the neuritis group.

TABLE 4—ELECTIVE LOCALIZING POWER OF STREPTOCOCCI FROM MYOSITIS AS COMPARED WITH STREPTOCOCCI FROM OTHER SOURCES

Source of Streptococci	Strains	Animals	Percentage of Animals Showing Lesions in												
			Muscles	Joints and Ligaments	Nerves	Stomach and Duodenum	Gall bladder	Appendix	Kidneys	Lungs	Skin	Pericardium	Myocardium	Endocardium	Central Nervous System
Myositis	28	202	80	33	9	13	3	1	9	7	4	4	17	8	?
Ulcer of stomach	37	168	4	12	0	68	21	1	3	0	0	2	3	10	?
Cholecystitis	12	41	7	17	0	29	80	0	5	5	2	0	2	10	?
Appendicitis	17	71	12	29	0	11	1	70	0	0	0	0	9	21	?
Acute poliomyelitis	22	123	16	15	4	13	2	2	2	11	0	5	7	7	46
Miscellaneous	71	212	12	9	4	9	1	1	9	7	4	0	4	12	?

In Table 4 is summarized the total incidence of lesions in the three groups of cases of myositis, and, for purposes of comparison, the incidence of localization in similar experiments in ulcer of the stomach, cholecystitis, appendicitis, poliomyelitis and of streptococci from similar sources from persons having miscellaneous diseases, and from normal persons. Among the diseases studied in the miscellaneous group were acute myelogenous leukemia, urticaria, ulcerative colitis, bronchitis, elephantiasis, thyroiditis, diabetes, purpura, jaundice, epididymitis, acute pyorrhea, dermatitis and angina pectoris. The incidence is given in percentages, so that the figures are directly comparable. Thus, of 202 animals injected with twenty-eight myositis strains, 161 (80 per cent) developed lesions in muscles, and sixty-seven (33 per cent) developed arthritis, manifested by turbidity of joint fluid or by hemor-

rhages in the periarticular ligaments. The total incidence of lesions in the muscles in each of the other diseases studied, and in the miscellaneous group, is much lower, varying from 4 per cent in ulcer to 16 per cent in acute poliomyelitis. The high figure in each of the other diseases corresponds to the disease the patients were suffering from at the time of the experiments. Thus, in ulcer, 68 per cent of the 168 animals developed lesions in the stomach or duodenum, in cholecystitis, 80 per cent of forty-one animals developed cholecystitis, in appendicitis, 70 per cent of seventy-one rabbits developed lesions in the appendix, in acute poliomyelitis, 46 per cent of 123 animals developed lesions in the central nervous system. This is in sharp contrast to the results obtained following the injection of 212 animals with seventy-one strains from miscellaneous sources. In these, as shown in the last line in Table 4, no particular organ was attacked. The incidence of lesions of 12 per cent in voluntary muscles is about that shown in Henrić's¹⁶ tables following injection of plated streptococci from miscellaneous sources. The myocardium in the myositis group had lesions, often marked, in thirty-four (17 per cent) of the animals, which, as might be expected, is a considerably higher incidence than occurred following the injection of other strains, in the case of ulcer it was 3 per cent, in cholecystitis, 2 per cent, in appendicitis, 9 per cent, in poliomyelitis, 7 per cent and following miscellaneous strains, 4 per cent.

These percentages, striking as they are, do not express adequately the differences observed at necropsy, because one lesion found in an animal injected with a nonmyositis culture counts for as much in the table as many lesions in an animal following the injection of the myositis strains.

In two cases in which there was a history of peridental symptoms (Cases 567 and 913), lesions of pulps of teeth or adjacent periosteum occurred in 50 per cent of thirty-five animals injected. The remarkable tendency of these strains to localize electively (Table 4) was paralleled in individual instances in which localization in animals was not only in muscles, but chiefly in the same group of muscles, as was noted in the patient. Thus, in myositis of the gluteus muscles (Case 621) the localization was chiefly in this group. In two cases of cervical myositis (Cases 567 and 909), the chief localization occurred in the muscles of the neck. In two cases of intercostal myositis, the localization was almost limited to the intercostal muscles, and in one case this was true in two sets of experiments (Fig 2, III and IV). In the case of lumbar myositis (Case 624) the lesions in the rabbits were chiefly in the lumbar muscles (Fig 2, II), a finding which supports the idea that infection may play a part in the etiology of chronic lumbago. In Case 913, in which the chief complaint was referable to the periarticular

16 Henrić, A. T. The Specificity of Streptococci, *J. Infect. Dis.* **19**: 572, 1916.

structures of the right knee, the striking localization occurred in animals around the knee joints (Fig 10, IV) The lesions in the muscles of the animals injected with the bacteria from the myositis-neuritis group were frequently situated around nerves (Fig 2, I, III and Fig 3, I), and the lesions in nerves independent of muscle lesions sometimes corresponded to those involved in the patient This was particularly true in Case 567 (Table 3), in which there were recurring attacks of facial neuralgia due to pulpitis and dental neuritis, and cervical myositis The affinity of the slightly hemolyzing streptococcus isolated from the foul dental pulp and the excised muscles of the neck for dental pulps and dental nerves, and muscles of the neck, was so marked that lesions in these structures developed even after intraperitoneal injection Besides lesions in muscles, arrhythmia of the heart, with lesions in the bundle of His and in the myocardium, developed in animals following the intravenous injection of streptococcus from the pyorrheal pockets in Case 601, the patient who had had repeated attacks of muscular rheumatism and who was suffering from attacks of syncope due to a damaged His bundle and impaired myocardial conduction Localization in muscles occurred in experiments performed during repeated attacks in some patients, for example, three times each in Cases 622 and 912, and five times in Case 1,035 In one patient (Case 912) localization in muscles of animals occurred as the chief lesion during each of three widely separated attacks of acute localized myositis Lesions in the gallbladder, myocardium and pericardium, without muscle lesions, were produced during an attack of cholecystitis and pericarditis, with dilatation of the heart, and lesions in the stomach or duodenum developed in the animals injected during two attacks of indigestion There were few or no lesions in the animals injected in the same way while the patient was free from symptoms on three widely separated occasions

The strains from generalized myositis tended to produce numerous small lesions in many muscles, sometimes thousands of lesions, while those from localized myositis tended to produce a few localized areas of muscle involvement

The objection which has been raised regarding dosage has been met not only by injecting smaller doses of cultures, but by directly injecting salt solution suspensions of the small number of bacteria in the pus expressed from tonsils Indeed, some of the most specific localizations occurred in these experiments It was thought worth while, therefore, to summarize the results obtained by injecting the small doses of the bacteria which had grown in the focus itself

Lesions in muscles developed in all of fourteen rabbits injected with the pus from five patients in the uncomplicated myositis group Two rabbits developed arthritis, and one rabbit had a hemorrhage of the

stomach in addition. Of the fifteen rabbits injected with the tonsil pus from nine patients in the myositis and arthritis group fourteen developed lesions in the muscles, ten in the joints, two in the stomach, two in the myocardium, and one in the endocardium. Of the six rabbits injected with the tonsil pus from five patients in the myositis and neuritis group, five developed lesions in the skeletal muscles, one in the stomach, two in the myocardium, and three in the nerves. Thus, of the total of thirty-five rabbits injected, thirty-three (94 per cent) had lesions in muscles. The results obtained following injection of the tonsil pus from twelve persons who were well at the time or who

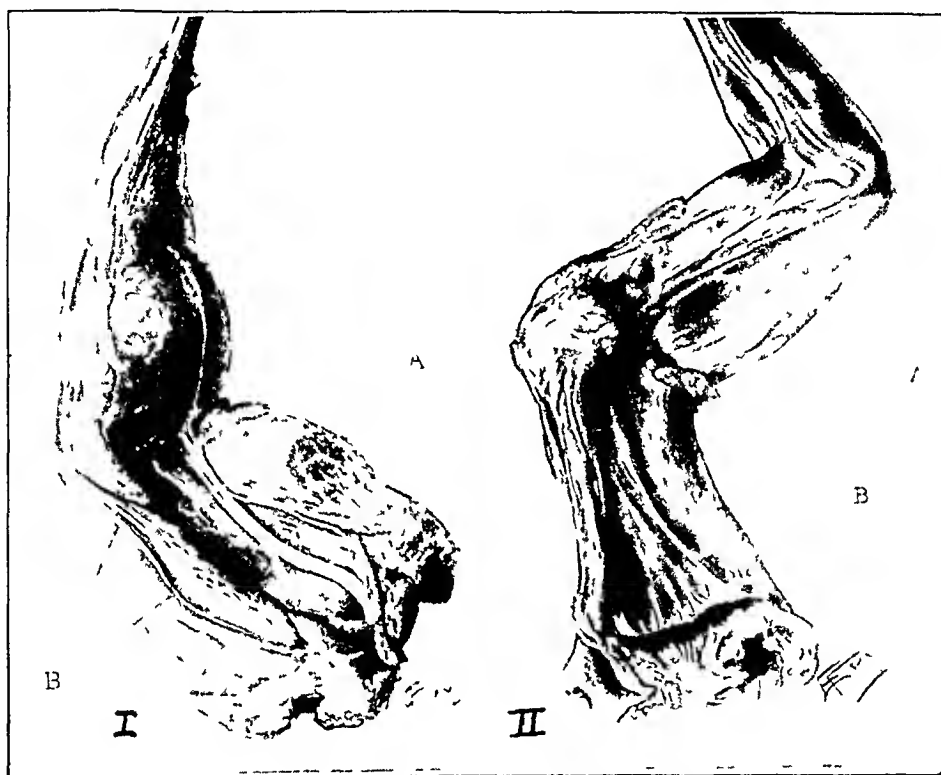


Fig 1—(I) Numerous small hemorrhagic lesions in the muscles of the hind leg of a rabbit forty-eight hours after injection of the primary culture of the streptococcus from the tonsils in a case of acute cervical and intercostal myositis (Case 909). (II) Numerous whitish necrotic lesions of the muscles of the fore leg and shoulder of a rabbit eight days after injection with the streptococcus from the tonsils after one animal passage in a case of marked but chronic myositis (Case 904).

had some ailment not attributable to infection were in sharp contrast. Of the twenty-four rabbits injected, only three (12.5 per cent) had lesions in muscles, and one rabbit had hemorrhages of the stomach.

The virulency of the bacteria injected was of a low order. Of a total of 202 animals injected (168 rabbits, seventeen dogs, nine guinea-pigs, seven mice, and one monkey) only forty-six died. 156 were anesthetized for examination. The results of cultures of the blood

made at necropsy were recorded in 144 instances. No growth was obtained in eighty-four, sixty cultures were positive. *Streptococcus* was found in forty-five, *staphylococcus* in nine, *colon bacillus* in eight, and a gram-staining bacillus in five. In most instances, the cultures were pure, although mixtures of *streptococcus* and *staphylococcus* were found occasionally. Cultures of the blood of the animals that were sacrificed remained sterile in 62 per cent and showed growth in 38 per cent. The blood of the animals that died showed growth in 54 per cent and remained sterile in 46 per cent. The number of colonies of streptococci on blood-agar plates in the positive cultures was relatively small. Often no growth occurred on aerobic plates when the deep broth cultures were positive. This was true even though the amount of blood inoculated was comparable in the two mediums. The oxygen sensitiveness was found also in the strains of streptococci isolated from the lesions in the muscles. Control culture of blood, and of various tissues without lesions including normal muscles, were often sterile when cultures from the lesions in the muscles revealed streptococci. But even the lesions resulting from a single injection tended to become sterile so that cultures had to be made soon after injection, preferably in from twelve to forty-eight hours, in order to demonstrate the presence of living bacteria.

REPORT ON CLINICAL AND EXPERIMENTAL OBSERVATIONS
DETAILS OF THE MORE IMPORTANT CASES IN THE UNCOMPLICATED
MYOSITIS GROUP (TABLE 1)

Case 1—Mrs O-E R [Case 130], aged 21, a farmer's wife, had had an attack of tonsillitis and puerperal septicemia two years before examination, and about the same time had noticed lumps in her neck. A few months before she came to the clinic the lumps had grown larger and more painful.

Examination revealed small, hard nodules in the right lobe of the thyroid, a chain of smaller lumps running down under the clavicle, lumps in the right axilla, and calcifying nodules along the biceps of the left arm. There was spasm of the left sternocleidomastoid. Operation was performed, and the calcifying masses which were found lying in the sternocleidomastoid muscle and extending out on each side of the muscles were removed from the right cervical region. The pathologist reported myositis ossificans.

A portion of the excised muscle was cultured Aug 21, 1914. The glucose-agar-shake culture yielded twenty-four small white colonies of a pleomorphic streptococcus, which produced slight and hazy hemolysis on a blood-agar plate. This tube was layered with liquid petrolatum and preserved in the dark at room temperature for subsequent studies.

Subcultures in tall columns of glucose broth from single colonies in the shake culture were injected intravenously into animals on different dates. The first series of animals injected (September 14-18) consisted of ten rabbits, five dogs and three guinea-pigs. Eight rabbits and two dogs had localization in muscles. The primary culture of the streptococcus from the muscle of one rabbit and one dog was injected into seven rabbits and one dog. Five of the rabbits developed lesions of muscles, the dog developed acute hemorrhagic pancreatitis.

The primary culture of the streptococcus from two of the rabbits was re-injected into two rabbits (third animal passage) One had slight muscle lesions, the other died of overwhelming infection without focal lesions

April, 1915, the second series of animals, consisting of three rabbits and two dogs, was injected with the subculture from a single colony in the agar tube Two of the rabbits developed lesions in the skeletal muscles, myocardium and joints, and one had no lesions Both dogs had hemorrhagic lesions of the flat muscles around the shoulders and thorax

March 8, 1916, a rabbit and a monkey were injected with the broth culture from the growth in the original agar culture Both developed hemorrhagic lesions in muscles and the monkey also had hemorrhages in the myocardium

At this time, the streptococci from 300 c.c. glucose broth were dried and placed in a desiccator over calcium chloride and kept from light After the subculture was dried, it revealed a pure growth of the streptococcus The one rabbit injected with a suspension in water developed a few small areas of hemorrhage, and infiltration of the flat muscles and fascia of the lateral aspect of the abdomen One of these areas surrounded a small subcutaneous nerve The



Fig 2—(I) Hemorrhagic lesions in the muscles of the neck surrounding the exit of the fifth cervical nerve of the rabbit injected with the tonsil pus in Case 909 (II) Localized area of hemorrhage, edema, and infiltration of the muscles of the lumbar region in a rabbit injected with the streptococcus from the tonsils in a case of lumbar myositis (Case 624) (III) Hemorrhagic lesions in the external aspect of the muscles of the upper thorax, especially around the exit of the small nerve trunks, in a rabbit injected with the streptococcus isolated from the mucopurulent discharge from the nose of a man at the time of an attack of intercostal myositis (IV) Hemorrhagic lesions in the internal intercostal muscles of the thorax in the rabbit referred to in Figure 2 (III)

dried streptococci were kept until November 1917, when it was found that cultures were negative The effect of the dead organism suspended in salt solution was then tested by injecting two rabbits intravenously Both developed hemorrhages in the flat muscles around the chest and shoulders and in the tendinous end of the muscles of the extremities

Of the thirty-eight animals injected in this case (twenty-six rabbits eight dogs, three guinea-pigs and one monkey) twenty-nine (76 per cent) had lesions in the skeletal muscles Ten had myocarditis The incidence of lesions

in other organs was low (Table 1) The fact that this organism lived and held its specific infecting power for so long (nineteen months) in the original shake culture, and the power of the dried dead streptococcal bodies to produce muscle lesions three years and four months after isolation, is nothing less than remarkable

CASE 2—Mr C G S [Case 624], aged 48, eight years before examination had had a severe attack of tonsillitis, followed by a "dull aching pain," soreness and stiffness in the muscles of the back, which had been constant A year previous to examination he was in bed for eight weeks with sciatica and lumbago The patient had two sisters who were troubled with rheumatism

The teeth were vital and free from pyorrhea The roentgenogram did not reveal apical abscesses, and the roentgenograms of the lumbar spine were negative The clinical diagnosis was chronic lumbar myositis and chronic tonsillitis The tonsils were large and contained pus and caseous material Tonsillectomy was performed The emulsion of the extirpated tonsils and the primary culture were injected, respectively into one rabbit each Hemorrhagic lesions in muscles, most numerous in the hip and leg muscles of the lumbar region (Fig 2, II), developed in both There were no lesions elsewhere One year and six months later, the patient reported that there was marked improvement and that shortly after the tonsils were removed the pain in the back almost disappeared Four years later he reported that except for slight distress at times he was free from his former pain, soreness and stiffness in the muscles of the back, and was feeling well

CASE 3—Mr S K [Case 904], aged 34 an ice deliverer had a severe exposure in the ice house, November, 1916, which was followed by intense pain in the back, extending to the legs, the attack was not preceded by any chill or fever January, 1917, the patient had another attack with pain in the neck, back, arms and legs, again not preceded by chills or fever He was taken to a hospital, and with daily application of hot packs for three weeks there was some improvement in his condition

On examination May 9, 1917 all his muscles and joints were stiff and very painful the pain being most severe in the muscles of the shoulders and arms There was an eosinophilia of 47 per cent The examination for trichina and the examination of the viscera were negative

Pus was expressed from the depths of the tonsils and cultured in ascites-glucose broth Plating showed an almost pure culture of distinctly hemolyzing streptococcus The growth in glucose-brain broth was centrifuged and suspended in salt solution and the growths from 7.5, 15 and 30 c.c. respectively was injected intravenously into three young rabbits After forty-eight hours, the rabbit which had received the growth from 30 c.c. died and a necropsy was performed while the body was still warm The rabbit which had received the growth from 15 c.c. was sensitive to the touch It had a convulsion, from which it recovered, and was then sacrificed The rabbit which had received the growth from 7.5 c.c. appeared well and was sacrificed On postmortem examination all three of these rabbits had small white linear lesions in the muscles, and in each rabbit these lesions were definitely more numerous in the muscles of the upper arm and shoulder than in other parts, their number was roughly in proportion to the number of bacteria injected The rabbit injected with the smallest dose had lesions of the skeletal muscles only and gave a sterile blood culture All organs in the rabbit injected with the 15 c.c. dose were normal grossly although microscopically the liver showed areas of slight infiltration Cultures of the blood yielded streptococci The muscle lesions consisted of dense leukocytic infiltration The rabbit which received the 30 c.c. dose had lesions in the heart muscle and in the nerve sheaths, in addition to the numerous linear lesions in the skeletal muscles There were no macroscopic lesions in the heart valves stomach or kidneys, and the knee joint fluid

was normal. Cultures of the affected portion of the muscles in ascites-glucose broth in twenty-four hours yielded an apparently pure culture of streptococcus which, when plated on blood-agar, developed a fine, green-producing type of colony in large numbers, and a few staphylococcus colonies.

Two rabbits were injected with broth culture from the muscle. One, which had received 3 c.c., died at the end of forty-eight hours and a necropsy was performed before rigor mortis set in. A number of lesions were found in the muscles, most numerous in the triceps, deltoid and the muscles of the shoulder blade, areas of hemorrhagic infiltration were present in the periosteum and in the upper epiphysis of the right humerus and a large number of embolic areas were noted in the kidneys, both in the cortex and in the medulla. Microscopic examination of the kidneys and muscles revealed sharply defined areas of dense leukocytic infiltration, with destruction of adjacent tissue. The second rabbit was injected with 3 c.c. of the same broth culture every second day for six days. On the eighth day it was sacrificed. The first day after the injection it was inactive, but apparently had no pain. On the second day, it was



Fig. 3—(I) Hemorrhagic lesions in the muscles, surrounding the nerve trunks, of the under surface of the scapula and thorax of a rabbit injected with the streptococcus from infected teeth of a man having myalgia, especially of the chest and shoulder muscles (Case 923). (II) Hemorrhagic lesions in the internal aspect of the muscles of the hind leg along the tendinous partition and adjacent nerve trunk in a rabbit injected with the streptococcus from the tonsils of a neurasthenic young woman at the time of localized myalgic pains of the thorax, and numbness of the right arm (Case 571).

better, but seemed sensitive. The next day its movements were slow and it showed a decided disinclination to walk. This tendency increased, until on the sixth day it refused to move unless shoved. On postmortem examination many white lesions were found generously distributed through the trunk muscles, and especially numerous in the adductor group of the thigh, shoulder and deltoid. Culture of the knee joint fluid yielded streptococci. The blood and kidneys were sterile. Microscopic examination showed lesions in the muscles, kidneys and liver consisting of areas of young scar tissue containing endothelial cells, large and small lymphocytes and fibroblasts but no leukocytes. There were areas showing moderate calcification.

On the basis of the above findings the patient's tonsils were removed. An emulsion was made of them and 2 cc were injected into a rabbit. This rabbit developed arthritis of one knee and lesions in the muscles and the nerve sheaths.

Agglutination tests were made with the patient's serum against the culture of the patient's tonsils, the streptococcus growth from one of the rabbit muscle lesions, and the staphylococcus growth from the muscle lesions of the patient and one of the rabbits, but only negative results were obtained.

Marked lesions of the skeletal muscles were produced in four rabbits injected with the streptococcus from the tonsils of this patient. The animals receiving the larger doses had a few lesions in other tissues (Table 1), while those injected with minimal doses had lesions only in the skeletal muscles. The lesions in the muscles were more numerous in the shoulders and arms than elsewhere. Slightly hemolyzing streptococci were recovered from the muscle lesions in one of the injected rabbits, and produced marked lesions in the muscles of two rabbits into which the culture was injected.

Three weeks after the removal of the tonsils, the patient was decidedly better and was able to walk with ease. After two months, he wrote that he was still sore in the shoulders, but that otherwise he was well and free from pain, although there was still some weakness in the legs. Three months after the operation, he complained of not being so well and was sent a vaccine, which consisted of the streptococcus recovered from the tonsils and from muscle lesions in the rabbits. Three years and nine months later, he reported that he had been free from rheumatic trouble, but that he continued to be nervous and very weak. In view of the destructive lesions which the streptococcus from this patient produced in the muscles of rabbits, the persistent weakness is believed to be due to actual destruction of muscle fibers and the formation of connective tissue.

CASE 4—Mrs P. L. [Case 909], aged 26, had an acute attack of follicular tonsillitis caused by the hemolytic streptococcus. Ten days later, the fever returned, accompanied by malaise, severe aching and general pain, at first associated with stiffness, and later with sharp pain and tenderness in the muscles of the left side of the neck, and a painful, tender area in the intercostal muscles above the left costal margin. The patient recovered promptly from this attack, but remained nervous and had mild attacks of pain in the muscles until seven months later, February, 1918, when her tonsils were removed. On dissection, a number of encapsulated abscesses were found in the tonsils. The patient has had better health than before and has been quite free from myositis.

The results of the animal experiments in this case were most interesting. The one rabbit injected with a small amount of pus expressed from the tonsil at the height of the attack of myositis was sensitive to touch and disinclined to move for two days. It was then chloroformed and numerous small hemorrhagic lesions in the muscles of the left side of the thorax, the inner aspect of the right thigh, and the lower end of the muscles of the right leg were found. The rabbit injected with the primary culture of the pus in ascites-glucose broth was extremely muscle-sore and weak when anesthetized forty-eight hours later. It had numerous small hemorrhagic lesions in the muscles of the extremities (Fig 1, I), and large edematous hemorrhagic lesions in the deeper layers of the muscles of the neck. A few small lesions were found in the kidneys, papillary muscles of the heart, and cordi tendinae. The blood of both rabbits was sterile. The blood-agar plate of the pus from the tonsil showed chiefly green-producing and indifferent streptococci, and a few colonies of hemolytic streptococci. One rabbit was injected with the broth culture in glucose broth from single colonies of each of the three types of streptococci. All rabbits remained well and all were free from lesions of muscles. The rabbit injected with the hemolytic streptococcus developed suppurative arthritis.

Stab cultures of the pus from the tonsil were made in tall tubes of ascites-tissue agar. This was incubated for nine days and then preserved in the dark for subcultures for further animal experiments.

The twenty-four hour cultures in tall columns of ascites-glucose broth from the bottom of the agar stab were injected (from nine days to three weeks later) into five rabbits. All had lesions of the muscles, but the lesions were fewer in number and less marked than those following injection of the primary culture. The one rabbit injected with the growth after an additional transfer in ascites-glucose broth had lesions in the tendinous portion of the muscles around the legs and shoulders. The blood-agar plates made from the broth cultures in all of these animals contained green-producing and indifferent colonies of streptococci, but no hemolytic streptococci.

Seven months later, when the patient was free from myositis, two rabbits were injected with the primary culture in ascites-glucose broth of the pus expressed from the tonsils. Both remained well and both were found free from lesions at necropsy forty-eight hours after the injections.

CASE 5—A young woman [Case 912], a laboratory technician, has been under observation for a number of years. She is a hard, persistent worker and her general health has been good, but she has had recurring attacks of

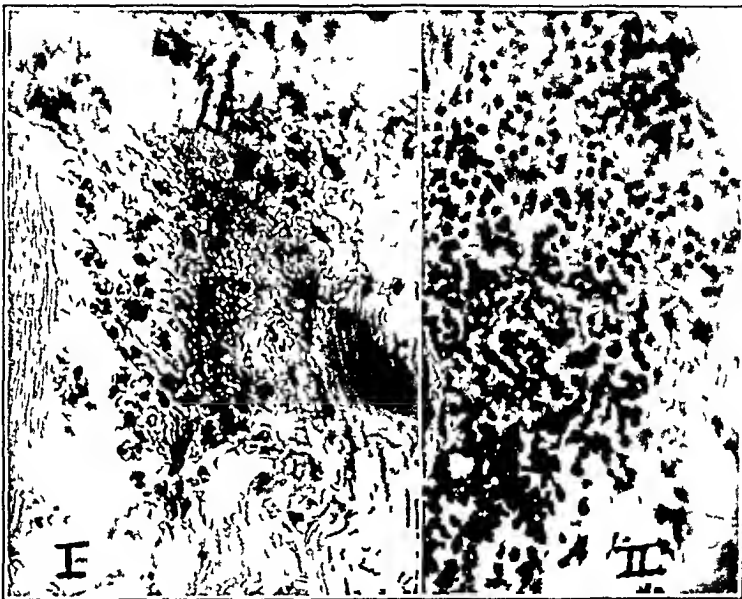


Fig 4—(I) Hemorrhage and leukocytic infiltration within and between the muscle fibers in the excised muscle in a case of acute rheumatic myositis in man. Hematoxylin and eosin (X 200). (II) Marked leukocytic infiltration in an excised section of muscle from the forearm of a patient with acute localized myositis. Hematoxylin and eosin (X 200).

mild localized myositis, several attacks of indigestion resembling ulcer or cholecystitis, and one severe attack of cholecystitis and pericarditis and myocarditis. In the winter of 1911, she developed severe pain in the intercostal muscles of the right lower chest, which persisted for some weeks and then gradually disappeared. No experiments were performed during this attack.

May, 1917, while the patient was well, four rabbits were injected as controls for some experiments in Case 913. Two rabbits were injected with a salt suspension of the pus expressed from the tonsils and two with the primary culture in tall columns in glucose broth. All the animals were well when chloroformed forty-eight hours later. The first two rabbits injected and one of the others the one receiving the small dose had no lesions. The rabbit receiving the large dose had a few small hemorrhages in the right biceps and in the intercostal muscles on the left side and a few around the left knee joint.

Nov 27, 1917, the patient developed pain in the muscles of the left arm and the right side of the abdomen. This came on without sore throat or ton-

sillitis, lasted for a few days, and then disappeared. On the first day of the attack, one rabbit was injected with an emulsion in salt solution of pus expressed from the tonsils. It was muscle sore when anesthetized the next day. Examination revealed a moderate number of small hemorrhages in the intercostal muscles on the right side, and the muscles of the scapula on the left side, and a large diffuse area of hemorrhage and edema in the subcapsular muscles on the right side.

Two weeks later, December 11, the patient developed, from no apparent cause, a sharp attack of indigestion. There was a feeling of fulness, distress and pain in the epigastrium for several days. Symptoms of hyperacidity developed from three to four hours after meals, which lasted for several weeks and then disappeared. At the height of this attack one rabbit was injected with pus expressed from the tonsils, and one with the primary culture in glucose broth. Both animals seemed to be well when chloroformed forty-eight hours after injection. The first rabbit had a hemorrhage in the pyloric end of the stomach and a few small hemorrhages in the intercostal muscles on the right side. The second rabbit had an ulcer in the pyloric end of the stomach, and whitish lesions in the gallbladder.

Feb 23, 1918, while the patient was feeling perfectly well, one rabbit and one dog were injected with the primary culture from the tonsils for controls in Case 1,035. Both animals were well when chloroformed forty-eight hours later, and had no lesions.

February, 1919, the patient had an attack of sore throat followed by myositis involving several shoulder and intercostal muscles. At the height of the attack a suspension of pus from the tonsils and the primary culture in broth were injected into one rabbit each. The rabbit injected with the first culture had scattered, large and small hemorrhages in the muscles throughout the body, the one injected with the second culture had a group of hemorrhages in the muscles of the thigh on the left side.

August, 1919, the patient became acutely ill with pain in the region of the heart, and some days later with pain in the region of the gallbladder. She had fever and leukocytosis. Pericardial friction rubs, dilatation of the pericardial sac and myocardium and acute tenderness in the region of the gallbladder developed. She was in bed for four weeks and then gradually recovered completely.

Two weeks after the onset of this illness two rabbits were injected with the primary culture from the tonsil. Both animals appeared to be well four days later when they were chloroformed and examined. Pericarditis, evidently secondary to focal necrotic lesions in the myocardium was found in both, and hemorrhage of the heart valve was found in one rabbit. There were no lesions of the stomach or gallbladder.

When the patient's convalescence was well advanced, a roentgen-ray examination was made of her mouth. The devitalized crowned second right upper molar showed slight rarefaction at the apices of the roots. This tooth was extracted and the primary culture in glucose-brain broth from the apical end was injected intravenously into two half-grown rabbits. Both animals were apparently well when anesthetized two days later. Both had embolic whitish areas in the gallbladder, surrounded by hyperemic or hemorrhagic zones, and edematous and hemorrhagic pulps of teeth. One rabbit had, in addition, a few whitish hemorrhagic areas in the left ventricle. There were no lesions in the stomach or muscles.

After the extraction of the tooth, the only one showing pathology, and the removal of the tonsils, Sept 30, 1919, on the basis of the experimental findings, the patient was free from stomach and gallbladder attacks, as well as from myositis, until Feb 4, 1921 when she developed soreness in the back part of the throat and symptoms of acute tonsillitis. On examination, a rather large mass of red, tender tonsillar tissue containing a decided crypt was found at the base of each tonsillectomy scar. Following this attack, she devel-

oped marked tenderness and swelling of the cervical glands immediately adjacent to the tonsillar area, a tender spot in the left deltoid, soreness in several places in the muscles of the back and a very tender and painful area in the muscles of the anterior outer and lower third of the left thigh. The tenderness in the muscles disappeared in four or five days and recovery was complete.

Three days after the onset of pain in the muscles of the left thigh a small amount of pus was expressed from the hypertrophied tonsillar tissue on both sides. The cultures on blood agar directly as well as from the primary culture in glucose-brain broth, contained large numbers of hemolyzing streptococci. Two rabbits were injected with the suspension of the pus, one from the right, the other from the left tonsil. Both developed necrotic and hemorrhagic lesions of muscles, but no lesions elsewhere. The primary culture in glucose-brain broth from each tonsil was injected into two rabbits each. These also developed localized areas of hemorrhage and necrosis in muscles chiefly of the flat muscles or tendinous portions. One rabbit had also a few small hemorrhages in the acid-secreting portion of the stomach.

The primary culture in glucose-brain broth of the slightly hemolyzing streptococcus from the muscle lesions of one of these rabbits was injected into

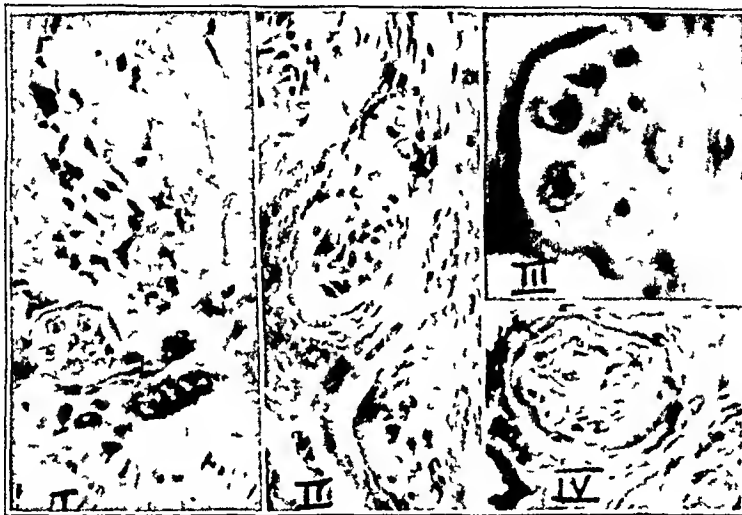


Fig. 5—(I) Section showing slight round-cell infiltration and complete plugging of a small blood vessel from hyperplasia of endothelial cells in the tendinous portion of an excised section of muscle in a case of chronic myositis and arthritis deformans. Hematoxylin and eosin (X 200). (II) Obliteration of blood vessels in the tendinous end of a muscle in a case of chronic myositis and peri-arthritis. Hematoxylin and eosin (X 200). (III) Obliteration of blood vessels from hyperplasia of endothelial cells in a muscle in a case of chronic myositis and arthritis. Hematoxylin and eosin (X 700). (IV) Obliteration of a blood vessel in another field of section shown in Figure 5 I. Hematoxylin and eosin (X 200).

two rabbits and the streptococcus from the blood of another animal was injected into one rabbit. The first two rabbits were well when chlorotomized forty-eight hours later. Both had lesions in muscles. The third rabbit died in twenty-four hours from an overwhelming infection with marked cloudy swelling of the muscles and viscera and a few localized hemorrhages in the muscles of the back.

As a control experiment on the importance of injecting bacteria from the focus of infection one rabbit was injected with a suspension in salt solution of the bacteria from the anterior part of the mouth and around the teeth

and two rabbits were injected with the primary culture of these in glucose-brain broth. The blood-agar plates contained a few hemolyzing streptococci, many staphylococci and *Micrococcus catenalis*. The rabbit injected with the suspension, which contained many times the number of bacteria contained in the tonsillar suspension, and one of the two animals injected with the primary culture, had no lesions, while the third rabbit had a few lesions in the flat muscles on the left side of the thorax.

About ten days after the attack of myositis, the patient, without indiscretion in diet, became nauseated and vomited several times during the night. She ate very little the next day because of distress in her stomach. For about a week after this attack, she complained of heaviness and distress, amounting to distinct pain, in the epigastrium, usually from two to four hours after meals. Sodium bicarbonate gave relief. There was no fever, no leukocytosis, and no tenderness in the region of the gallbladder. The gastric symptoms disappeared entirely in about ten days.

Two days after the beginning of this attack, one rabbit was injected with a small amount of secretion expressed from the tonsil tissue. The amount was so small that it did not produce noticeable turbidity in 2 cc salt solution in which it was suspended for injection. The rabbit appeared to be well when chloroformed forty-eight hours later. It showed a number of small hemorrhages in the cardiac end of the stomach, and no lesions elsewhere.

The following day two rabbits were injected, one with 25 cc, the other with 5 cc of the primary culture in glucose-brain broth from the tonsil tissue. Both appeared to be well when anesthetized two days later. Both had small hemorrhages in the stomach, most numerous in the pyloric end and along the lesser curvature. There were no other lesions.

An exactly similar experiment was performed at the same time as a control with material from the tonsil tissue from a patient having acute localized myositis (Case 1035). None of these three animals had lesions in the stomach, but all three had lesions in muscles.

In order to determine still further the importance of the peculiar infecting power which the bacteria seemed to acquire in a focus in the causation of symptoms in this patient, two rabbits were injected, one with the small amount of pus expressed from the tonsil, the other with the primary culture, two weeks after she recovered from the gastric attack. Both animals were well when chloroformed forty-eight hours later. There were no lesions in the stomach or duodenum, and none in the muscles with the exception of edema and a small group of hemorrhages, which had the appearance of a bruise, in the inner aspect of the knee in the one injected with the culture. This was in sharp contrast to the findings in four rabbits injected on the same day and with the same material from two cases (Cases 622 and 1035) of mild myositis, in which all of the animals had lesions of muscles, but no lesions elsewhere.

CASE 6—The patient [Case 1035], a laboratory technician, aged 20 developed a typical attack of stiff neck following an attack of tonsillitis in December, 1917. There were stiffness and pain on movement of the muscles of the right side of the neck, marked tenderness at the attachment of the muscles to the transverse process of the fifth cervical vertebra, and enlarged, slightly tender lymph glands just anterior to the margin of the right trapezius. There was no tenderness elsewhere and little or no fever. The tonsils were moderately enlarged, and a small amount of pus was expressed from the pole of each.

The pus, when plated directly, as well as the cultures in broth on plating, showed almost a pure growth of slightly hemolyzing streptococci. The emulsion in salt solution of a small amount of the pus, so small as to produce only slight turbidity, was immediately injected into a half-grown rabbit. The animal became lame in the right hind leg in two days, and was chloroformed. It had large swelling, edema and hemorrhage of the muscles of the anterior and outer aspect of the right tibia and along the tendo achillis around the

right ankle, and a moderate number of small hemorrhages in the intercostal muscles on the left side and in the deeper muscles on the left side of the neck. The joint fluids were clear, and no lesions of viscera or nerve trunks were found.

Two additional rabbits were injected at the time of this attack, one with the primary culture of the pus in glucose-brain broth, and the other with a pure culture of the streptococcus in this medium after plating on blood-agar. Within forty-eight hours both animals showed a marked disinclination to move about and were chloroformed. Both had marked, localized lesions, chiefly in flat muscles and tendinous ends, turbidity of joint fluid and hemorrhages around one knee joint, and a few embolic areas in the kidneys.

January, 1918 the patient had an attack resembling cholecystitis, which followed a severe fall on the right hip on the ice. February 17, the pain in the

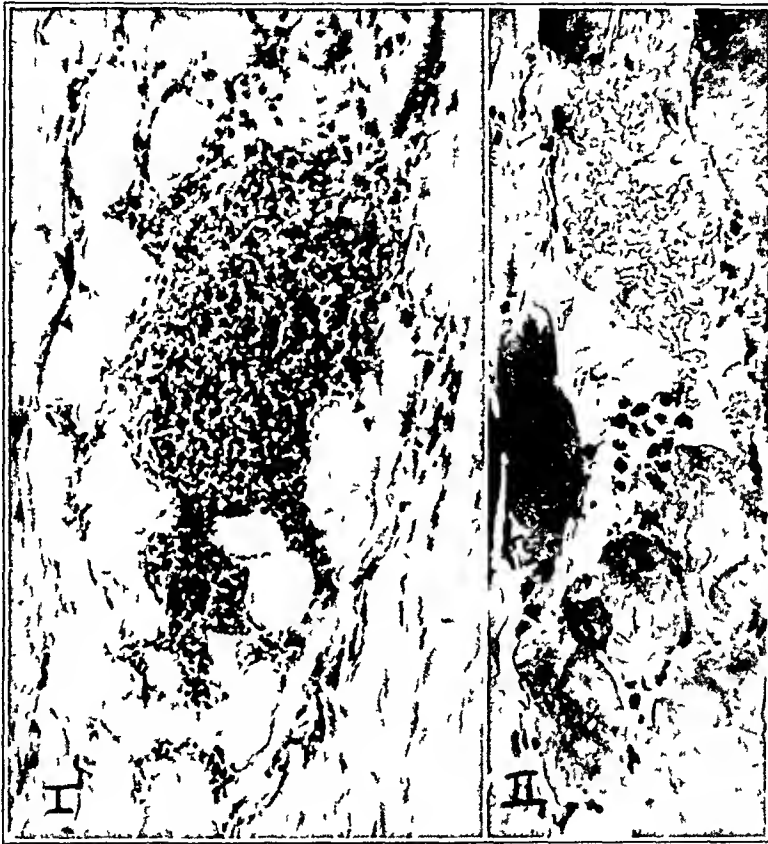


Fig 6—(I) Section of a whitish lesion, like those shown in Figure 1, (I), in the muscle of a rabbit injected with the streptococcus from the tonsils in Case 904. Hematoxylin and eosin (X 100). (II) Slight leukocytic infiltration and edema between the muscle fibers and the myocardium of a rabbit injected with streptococcus from the tonsils, after one animal passage, in a case of myositis and myocarditis (Case 601). Hematoxylin and eosin (X 200).

region of the gallbladder recurred during the night. Five days later, after she had completely recovered from the second attack of pain, two rabbits and one dog were injected with the primary culture from the tonsils in tall columns of glucose broth. The animals were chloroformed forty-eight hours later. No lesions were found.

April, 1920, the patient developed, from no apparent cause, pain and tenderness in the left arm, midway between the shoulder and elbow joint. She was nauseated and dizzy for two days. The left tonsil contained a large crypt filled with yellowish-gray, foul smelling, cheesy material. Firm pressure dis-

lodged the plug and a full minim of grayish pus escaped. A small part of the latter was suspended in 2 cc salt solution and injected into a medium sized rabbit. The rabbit was well when it was chloroformed the following day. It had a small hemorrhage in the tendinous end of the muscles of the posterior aspect of the left leg, and a large hemorrhagic area in the muscles of the anterior and inner aspect of the upper end of the right tibia.

On the basis of these experiments tonsillectomy was advised, and the tonsils were removed in July, 1920. The patient was free from the attacks resembling cholecystitis and from myositis until Feb. 1, 1921, when she developed pain in the muscles of the left arm and right scapula, following sore throat. Examination revealed infected tonsil tissue, with one deep crypt containing pus at the lower end of the tonsil scar on the right side. No experiments were performed at this time. The symptoms referable to the muscles disappeared in a few days, but the right tonsil area remained tender and painful on swallowing. Feb. 18, 1921, she developed a sharp pain, especially on exertion and pressure on the inner aspect of the muscles above the left knee, and the following day stiffness and soreness of the muscles of the deeper layers of the right side of the neck. This was not associated with fever or general illness, and the pain in the muscles lasted only two or three days. February 19, the small amount of secretion expressed from the tonsil tissue was suspended in salt solution and injected into a rabbit weighing 1000 gm. The animal appeared to be well forty-eight hours later when it was chloroformed. An area 1 cm in diameter in the muscles of the inner aspect of the left thigh just above the knee joint showed a group of hemorrhages, whitish lesions, edema and infiltration. Similar, but smaller, localized lesions were found in the muscles around the right elbow joint and the left side of the thorax. The two rabbits injected with the primary culture from the tonsil in glucose-brain broth appeared to be well when they were anesthetized forty-eight hours after the injection. Both animals had localized lesions in muscles, but no lesions elsewhere. The cultures of the tonsillar secretion on blood-agar, the primary culture in broth, and the culture from the muscle lesions contained green-producing streptococci and staphylococci.

February 28, the patient awakened with pain in the throat, which increased on swallowing, and during the day she developed pain over the left shoulder blade, which was aggravated on muscular effort. This disappeared in a few days. On the morning of March 1 while the pain in the muscles was still present, one rabbit was injected with a suspension in salt solution of the small amount of pus expressed from the remnant of tonsil tissue. The rabbit appeared to be well when it was chloroformed forty-eight hours later. Groups of hemorrhages in the muscles over the left shoulder blade and in the superficial fascia over the back were found. Late on the same day, one rabbit was injected with 5 cc of the primary culture of the tonsil pus in glucose-brain broth. The rabbit appeared to be well when it was chloroformed forty-eight hours later. A few small hemorrhagic lesions were found in the muscles of the left fore extremity, especially in the tendinous ends of the muscles around the elbow joints.

DETAILS OF THE MORE IMPORTANT CASES IN THE MYOSITIS AND ARTHRITIS GROUP (TABLE 2)

CASE 7—Mr. F. K. [Case 536], aged 27, came to the clinic, Nov. 24, 1915, complaining of rheumatism which he had had for three years. It began with pain in the right shoulder and right knee, later it extended to the left shoulder and left knee, and occasionally to the second finger of both hands. There was also aching of the muscles of the back, arms and legs. The trouble in the joints, except in the right shoulder, had stopped, but the pain in the muscles was constantly growing worse. Examination revealed peri-arthritis, and lesions chiefly of the tendinous ends of muscles. The tonsils contained much caseous material and were removed. After the removal of the tonsils, the

patient had less muscular pain. A year later he came back. The muscle aching had disappeared, but he had periodic attacks of muscle soreness associated with sleepiness and tired feeling. The roentgen-ray examination of the teeth was negative. The patient was not referred again to the laboratory, but was given general treatment and dismissed.

The rabbit injected with the pus from the tonsil seemed to be ill when it was chloroformed forty-eight hours later. Examination revealed a moderate number of edematous, necrotic lesions in the muscles of the extremities, neck and shoulders, a few hemorrhages in the papillary muscles of the left ventricle and in the tricuspid valve, and slightly turbid fluid in the knee joints. Cultures from the lesions in the muscles contained *Staphylococcus aureus*.

The two rabbits injected with the primary culture in ascites-glucose broth had marked lesions in the muscles and myocardium, and one rabbit had in addition a few embolic lesions in the kidneys, and turbid joint fluid. The primary culture from lesions in the muscle of one of these, containing staphy-

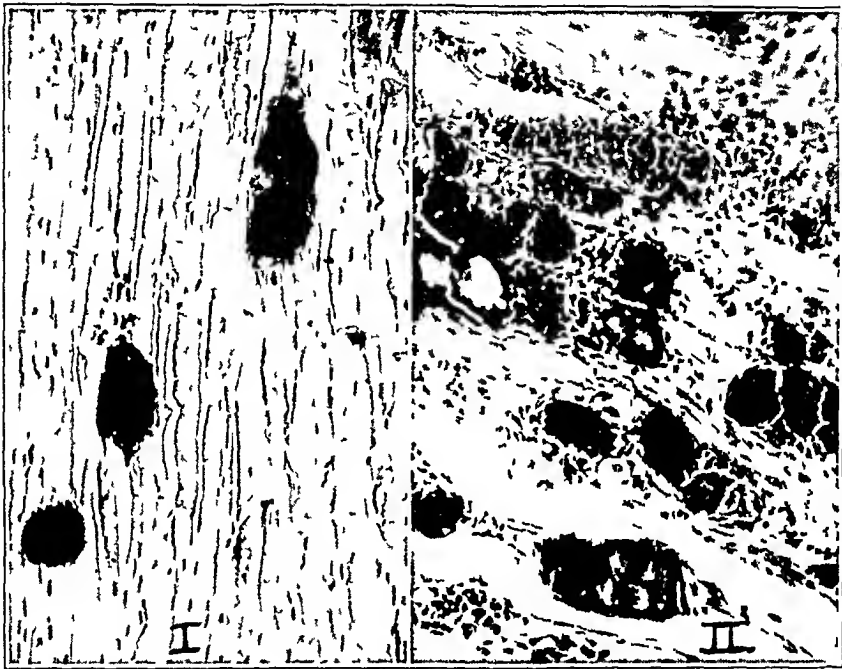


Fig 7—(I) Section of muscle in a rabbit six days after injection of streptococcus from the muscle in the case of calcifying myositis (Case 130). Note the marked deposit of calcium in sharply circumscribed areas. Hematoxylin and eosin (X 80). (II) Section of muscle of rabbits shown in Figure 1, I. Note the disintegration of the muscle fibers, leukocytic infiltration, and moderate deposit of calcium in the necrotic areas. Hematoxylin and eosin (X 100).

lococcus, was injected into one rabbit. It seemed muscle sore when it was chloroformed four days later. A few muscle lesions, multiple suppurative arthritis, a few embolic lesions in the kidneys, and cystitis with marked infiltration of the bladder wall were found.

CASE 8—A physician [Case 552], aged 51, had suffered from repeated attacks of muscular and neuralgic disturbances, lumbago and sciatica, for twelve years. He had had rheumatic fever when he was a child. He had always had more or less sore throat, which had been worse for the last three years. Three years before examination he had had pericarditis, and eighteen months before, following an attack of sore throat, he had had acute arthritis of the right knee, and pain in the muscles of the neck and left shoulder. Roentgenograms of the knee and spine were negative. Both tonsils contained a few small pus

pockets in their depths. The tonsils were removed and an emulsion was made of them and cultured. The growth of green and slightly hemolyzing streptococci which was obtained was injected into two rabbits, and produced in both animals hemorrhagic lesions in the muscles of the back and shoulders, and in one rabbit a few lesions in the thigh and arthritis of the knee joints (Table 2).

The patient has reported that within two months he became free from all rheumatic disturbances, and that he had gained in weight. Five years after his tonsillectomy he wrote that his former symptoms had not returned.

CASE 9—A middle aged physician [Case 622], had had repeated attacks of acute tonsillitis for many years. Following two such attacks, while he was in the medical school, hydrops of the left knee developed. He had repeated attacks of lumbago, usually with mild myositis, involving chiefly the flat muscles around the back, neck and thorax. Because of these attacks, and because foul smelling, cheesy plugs kept forming in the tonsils, tonsillectomy was performed. After this there was freedom from the symptoms for about two years, then followed a loss of endurance, a prolonged low grade arthritis which involved the small joints of the hands, a number of attacks of myositis, irritability of the bladder, presumably due to infection of the prostate, and a troublesome hyperchlorhydria. The joint and muscle symptoms became worse following inflammation of the tonsil tissue in the base of the left tonsillectomy scar. These symptoms, and those referable to the bladder, disappeared promptly after the removal of the remnant of the left tonsil, which contained a small abscess filled with foul smelling pus. The streptococcus from this abscess and from shreds in the urine produced arthritis, chiefly of the peripheral joints, and myositis in the four rabbits injected.

About two years later, the patient had an attack of myositis and subacromial bursitis, a typical attack of so-called "painful" shoulder, and the symptoms referable to the bladder returned. They were never severe, but persisted for a number of months, those caused by the bursitis were severe at first and were associated with tender deltoid and triceps muscles on the affected side. The symptoms grew milder during several months, and then persisted practically unchanged for nearly a year. The nine rabbits injected with the streptococcus from the shreds in the urine developed myositis and subacromial bursitis. A devitalized molar, believed to bear a causal relationship, was extracted, even though the roentgenogram was negative. A small granuloma was found situated so that its demonstration was impossible in the roentgenogram. The granuloma was incubated a short time, a pure culture of slightly hemolyzing streptococcus was isolated, and a large colony of streptococci was found near the apex in intimate relation to the blood vessels. The symptoms referable to the shoulder disappeared promptly, a result strikingly similar to the results reported by Evans in this painful affection. No joint or muscle symptoms developed until seventeen months later, when a mild attack of myositis occurred, and symptoms of hyperchlorhydria again became troublesome. Examination at this time revealed three devitalized teeth with defective root canal fillings. Two of these, the two left lower bicusps, had been devitalized eighteen years before, the third, a right lower molar, had been devitalized six years before. The roentgenogram of the first two showed increased density of the structures around the roots, and one showed a faint line of rarefaction over the apex, while the molar appeared to be normal. The material, which was removed in a sterile manner from the canals of the first two teeth, contained pure cultures of a green-producing streptococcus. Antiseptic treatments efficiently sterilized the canals, but it was feared that the tissues beyond could not be thus sterilized, and the three nonvital teeth were extracted under sterile precautions. Cultures of the apices showed streptococci in each. These cultures were injected into three rabbits. All three developed characteristic lesions. The results are illustrated in the following experiment.

A rabbit weighing 1,330 gm was injected intravenously Oct 5, 1918, with 8 c.c glucose-broth culture from the roots of the second left lower bicuspid October 6, at 7 30 a m, the animal was found extremely weak, scarcely able to walk At 10 a m it was found lying quietly on its side, and it died without convulsion Necropsy revealed a moderate number of hemorrhages in the muscles of the back and hips, and a large hemorrhagic, edematous area opposite the root of the right lower incisor The peridental membrane of the left lower incisor was edematous and hemorrhagic throughout, while that of the right was hemorrhagic and edematous at the apex The pupils were edematous and hemorrhagic The other teeth appeared to be normal There were a large number of submucous hemorrhages in the stomach and appendix and one hemorrhage in the tricuspid valve The joints were normal, as were also the myocardium, kidneys, spleen, liver and other organs The blood-agar plate of the blood contained no growth, while the blood-agar plate of the hemorrhagic



Fig 8—(I) Cross section of capillary showing aggregation of leukocytes within the lumen adjacent to the lesions in the muscle shown in Figure 6, (I) Hematoxylin and eosin (X 500) (II) Diplococcus in the wall of a capillary showing marked swelling of the endothelial cells in the muscle of a rabbit one day after five daily injections of liquor formaldehydi-killed streptococci from Case 904 after one animal passage Giemsa (X 1000) (III) Cross section of an arteriole and a venule in the muscle adjacent to lesions in rabbit shown in Figure 1 (I) Note the marked swelling of the endothelial cells Hematoxylin and eosin (X 500) (IV) and (V) Sections of normal arteriole, venule, and capillary of muscle in Figure 8 (III), but remote from lesions Note the absence of swelling of the endothelial cells Hematoxylin and eosin (X 500) (VI) Marked swelling of the endothelial cells in a capillary in the muscles of rabbit shown in Figure 1 (I) Hematoxylin and eosin (X 500)

pulp contained a moderate number of slightly hemolyzing and green-producing streptococci

Immediately following the extraction of his teeth, the patient developed a sharp attack of myositis of the muscles of the lower right thorax, after which there was a noteworthy freedom from symptoms for six months A similar attack, though milder, involving the muscles of the neck and shoulder, occurred

following infection of the gum, which became painful from pressure of an improperly fitting denture. With the correction of this difficulty, the myositis again subsided. Since then, the patient has had unusually good general health and has been quite free from myositis attacks and from symptoms of hyperchlorhydria.

During the latter part of February, 1921, the patient developed an attack of nasopharyngitis, followed by myositis of the muscles of the left lower thorax, the left shoulder, and the left side of the neck. During this attack two rabbits were injected, one with a suspension of the secretion from a swab from the nasopharynx, the other with the primary glucose-brain-broth culture. Both were well when chloroformed forty-eight hours later, and both had hemorrhagic lesions in muscles, chiefly of the thorax and shoulder.

The incidence of lesions found in the seventeen rabbits injected is given in Table 2.

Improvement followed the removal of foci of infection in this patient on four widely separated occasions, and the presence of bacteria in the foci removed, which had specific localizing power, was demonstrated on three occasions.

CASE 10.—A laboratory worker [Case 913] aged 37, had pain and stiffness in the left knee, noticeable in walking downstairs, or in rising from a sitting position. After nine months, this spontaneously disappeared. About two months later the right knee became affected to a greater extent, with increasing severity. The painful area extended up the muscles of the inner aspect of the thigh, and occasionally there was pain in the muscles around the shoulders and hip joints, and intermittent slight looseness of one or two teeth. Two ulcerated teeth had been extracted, but without beneficial results.

Examination revealed swelling and tenderness in the subpatellar bursa. The tonsils were red, but small. The anterior pillars were adherent and tightly covered a large part of both tonsils. They were uncovered with difficulty and four or five crypts were found packed with white cheesy material and pus, which was expressed.

Two rabbits were injected with the suspension in salt solution of this pus, and as a control two rabbits were injected with a suspension of a comparable amount of pus expressed from the tonsils of a person (Case 912) who was well at the time. The four animals appeared to be well when they were chloroformed forty-eight hours later. The two animals injected with the pus from the patient's tonsils had lesions in muscles, periosteum and joints. One animal had a few small red lesions in the tendinous portions of the muscles of the shoulder and the bursa just below the patella, a hemorrhagic area below the right lower cuspid, and a periostitis below the right eye. The other had edema of the tissues surrounding one knee joint, circumscribed areas of hemorrhage in the periosteum of the upper and inner aspect of the tibia just below the epiphyseal line, hemorrhagic lesions in the inner aspect of the periosteum adjacent to the elbow joint and in the capsule of the hip joint, a number of linear hemorrhages in the quadriceps tendons, and an area of hemorrhage in the muscle overlying the hip. The two rabbits injected with the pus from the tonsils of the person who was well did not show lesions.

The following day two rabbits were injected with the primary culture of the pus from the patient's tonsils, and two others were injected with identical amounts of the culture of the pus from the tonsils of the normal control. Smears and plating of both cultures revealed almost a pure, short chain, slightly hemolyzing streptococcus. The rabbit injected with the smaller dose (the growth from 15 cc) of the control culture did not develop lesions, the one receiving the larger dose (30 cc) had a few small hemorrhages in muscles and slight turbidity of joint fluid, which was sterile. Of the two rabbits injected with the culture from the patient, the one rabbit receiving the smaller amount developed hemorrhagic lesions, especially of the ligaments around the joints, the

subpatellar bursae and intracapsular fat, and the intercostal muscles, a hemorrhagic area in the stomach, and a few embolic streaks in the kidneys. The lesions in this rabbit were more pronounced than those in the control rabbit injected with the larger dose. The rabbit injected with the larger dose died of an overwhelming infection within eighteen hours, without developing focal lesions. A third rabbit was then injected with the same culture. It survived for forty-eight hours, and developed lesions in the tendinous portion of the muscles surrounding the right elbow, in the intercostal muscles, and in the body of the triceps.

As a further control of this work, cultures were made from single colonies taken from aerobic blood-agar plates seeded with the tonsil pus culture of the patient. Hemolytic and green-producing streptococci were used. The animals injected with these cultures gave negative results, which bears out our general finding that not every streptococcus will cause these muscle lesions, and that the streptococcus which will cause these lesions either does not grow or loses its peculiar infecting property when grown on aerobic blood-agar plates.



Fig 9—(I) Cross section of arteriole, venule, and nerve fiber of a rabbit one day after five daily injections of liquor formaldehydi-killed streptococci from Case 904. Note the marked proliferation of the endothelial cells in the arteriole. Hematoxylin and eosin (X 500). (II) Cross section of nerve fiber and terminal sensory organ in muscle of rabbit referred to in Figure 9 (I). Hematoxylin and eosin (X 500). (III) Diplococcus in partial focus within the swollen cells of the sensory organ shown in Figure 9 (II). Giemsa (X 1000).

The culture made from the muscle lesions which developed in the rabbit receiving 15 cc of the patient's tonsil pus culture was injected into three rabbits. Two rabbits received 2 cc doses of the broth culture and one a 5 cc dose. The first two rabbits had hemorrhagic lesions in the muscles. The third rabbit had a few lesions in the muscles and some in the periosteum.

On the basis of these findings, the tonsils were removed, and, although small, a number of abscesses were found in their depths. Injection into animals of a salt solution emulsion, and the resulting cultures, gave results almost identical with those obtained with the tonsil pus and cultures.

Altogether twelve animals were injected with organisms obtained either directly from the tonsils or by animal passage. One of these animals died of an overwhelming infection in less than twenty hours. All of the others had

lesions of the muscles of a uniform type, six also had lesions in the periosteum and six joint lesions of the subpatellar bursa. Of these eleven rabbits, one had, in addition, endocarditis, one had a stomach lesion, two had kidney lesions, two had skin lesions, and two had enlarged lymph glands (Table 2).

Four rabbits were injected with the material obtained from a normal person whose tonsils were in a worse condition than the patient's tonsils. Three of these rabbits were entirely negative. The fourth, which received more than twice as great a dose as any of the rabbits of the first series, developed a few lesions in muscles and periosteum, and a slight turbidity in the knee joint fluid. The organisms, although not highly virulent, showed evidence of being more virulent than those obtained from the normal control.

The patient's serum agglutinated specifically her own strain in the first and second culture generation and the strain from the muscle lesion in higher dilutions (1:100) than did the serum from the normal person, but not the control strain in the first and second culture nor the hemolytic streptococcus from her own tonsils.

A few days after the patient's tonsils were removed, the pain in the muscles and subpatellar bursa had disappeared almost completely, only a slight tenderness in the joint remained. After a few weeks there was some return of the trouble, but the patient recovered entirely within two years, and has remained well for several years.

DETAILS OF THE MORE IMPORTANT CASES IN THE MYOSITIS AND NEURITIS GROUP (TABLE 3)

CASE 11—Miss B. C., [Case 571] aged 22, had never been strong, and was very nervous. She had a dry hacking cough, sometimes raising blood-stained sputum, with pain in the muscles of the chest and around the shoulders, numbness of the right arm and nausea, especially when she coughed. She was constipated, suffered from gas in the bowels, and had had right-sided headaches since childhood. She had lost 10 pounds in weight in the last year, and weighed 98 pounds at the time of examination.

Physical examination revealed moderate hypertrophic rhinitis, hypertrophied adenoids, infected tonsils and a cloudy right antrum. Roentgenograms of the chest and the colon were negative. Nasal washes were prescribed, and tonsillectomy was performed. The patient improved markedly soon after the tonsillectomy. The excised tonsils were cut into small pieces and were found to contain pockets filled with foul smelling pus. The emulsified tonsil was cultured and a growth consisting mostly of streptococci was obtained. This was injected into four rabbits and one mouse. All of these animals developed muscle lesions, three chiefly in the muscles of the shoulders, fore legs and thorax, two of the rabbits had hemorrhage and edema in the sheath of the popliteal nerve (Fig 3, II).

CASE 12—A man, [Case 601] aged 46, a painter, had been subject to muscular rheumatism for several years. Since February, 1916, he had had attacks of pain across the chest in the region of the heart, radiating to the back, so severe that he felt like lying down. For a week previous to his examination, the pain had been more or less constant. He was short of breath, especially at the time of the attacks, and he fainted on several occasions. His habits were good.

At the time of examination his cheeks were flushed, his pulse 64, and irregular, there were no heart murmurs. He had marked pyorrhea, and the gum edges presented a bluish discoloration. There was no granular degeneration of the erythrocytes. The Wassermann reaction was negative. A cardiogram showed a pulse rate of 50, marked impairment of conduction, and a damaged right His bundle. The urine examination was negative. He had not had tonsillitis, and the tonsils were fairly clean. He had a slight laryngitis.

A culture of the pus from the pyorrheal pockets contained a moderate number of colonies of green-producing and slightly hemolyzing streptococci. Roentgenograms of the teeth revealed alveolar abscesses in three. These teeth were extracted, and prophylactic treatments for the pyorrhea were instituted. The cultures made from the pus from the pyorrheal pockets and the alveolar abscesses, both of which were extremely foul, contained a moderate number of colonies of green and slightly hemolyzing streptococci, a few gram-positive bacilli, and staphylococci.

Four and one-half months after the extraction of the teeth and the treatment for pyorrhea, the patient was reported to be feeling better, there was less dyspnea, but at times he still had palpitation and spells of weakness, although no fainting spells as formerly. Two years later the improvement was still manifest.

In this case thirteen animals were injected, either with the pus from the pyorrheal pockets or with the streptococci from the lesions produced on animal passage. The incidence of lesions is given in Table 3. Seven of these animals

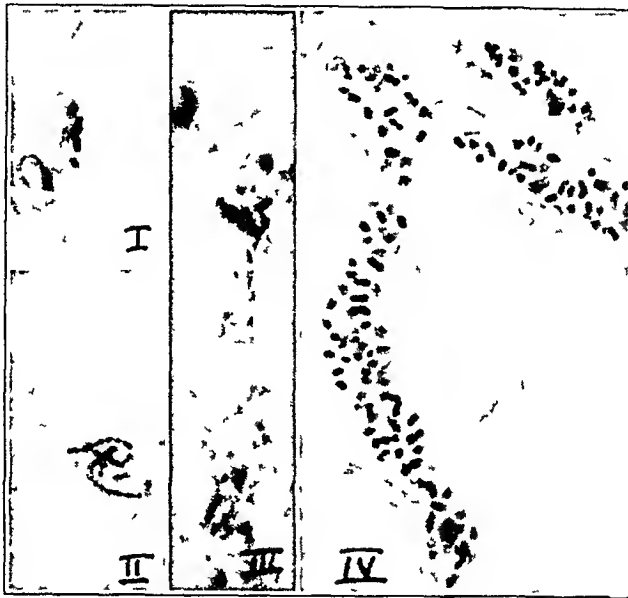


Fig. 10—(I) Chain of two diplococci in the section of human muscle shown in Figure 4 (I). Gram-Weigert (X 1000). (II) Chain of streptococci in the area of leukocytic infiltration shown in Figure 4 (II). Gram-Weigert (X 1000). (III) Diplococci in the area of leukocytic infiltration shown in Figure 6 (I). Gram-Weigert (X 1000). (IV) Large number of gram-positive diplococci in capillaries of a hemorrhagic area in the quadriceps tendon of a rabbit two days after injection with the streptococcus from the tonsils in a case of periartthritis and myositis (Case 913). Gram-Weigert (X 1000).

had lesions in the muscles. Ten (80 per cent) had pericardial, myocardial or endocardial involvement. One had a hemorrhagic lesion in the region of the bundle of His, one had a mural vegetation in the region of the bundle of His, and one, a dog, which developed a gallop rhythm of the heart, had a lesion in the left ventricular side of the septum just beneath the aortic cusp, probably in the left limb of the His bundle. Two of the animals had lesions in the aortic arch. One rabbit had a moderate number of hemorrhages throughout the length of the left vagus nerve. The right sympathetic nerve and the right cervical sympathetic ganglion were hemorrhagic and edematous. Sections showed a large number of gram-staining diplococci in the hemorrhagic ganglia. A few hemorrhages were found also in the cervical plexus.

The localization and lesions produced in this case were quite unique. The gallop rhythm, the lesions in the bundle of His and sympathetic nerves and ganglion are especially noteworthy.

CASE 13—Mr P W [Case 2291], aged 49 had had recurring attacks of appendicitis until his appendix was removed in 1910. He came to the Clinic Sept 18, 1918, complaining of nervousness, loss of mental and physical endurance, attacks of fluttering of the heart at night, pain in the back, and a daily afternoon rise of temperature. The pain in the back was dull, aching, and deep in the muscles of the lumbar region, often radiating into the thighs. The pain was worse when he exercised, he could walk only several blocks at a time. His temperature rarely went above 99.6 F. He had seen many physicians, some of them diagnosed incipient pulmonary tuberculosis because of the temperature and the finding of a few râles in the lungs, others suspected, but did not demonstrate, chronic focal infection. He had an attack of pneumonia lasting three days nine months before coming to the Clinic.

The man was well nourished, but markedly neurotic. The vasomotor tone was below par. The thyroid was palpable on both sides, indicating a small adenoma. The pulse was regular, and the heart tones were clear. A few râles were heard over the apices of both lungs. Blood culture and blood Wassermann reactions were negative. The blood pressure, the urine, the stool and the blood count were normal. The metabolic rate on three occasions was —23, —19 and —2, respectively. The tonsils appeared to be normal on inspection, but on pressure a moderate amount of pus was expressed.

One rabbit was injected with a suspension of a part of this pus, and on the following day another rabbit was injected with the primary culture in glucose brain broth. Both animals appeared to be muscle sore when they were chloroformed three days later. The first rabbit had a number of circumscribed areas of hemorrhagic edema in both psoas muscles. The lumbar nerves passed through some of these areas, and hemorrhages were found in the sheath of two of the nerves remote from the lesions in the muscles. Similar, but smaller lesions were found in the muscles in the lumbar region and thighs. The second rabbit showed almost identical findings, but the lesions were relatively more marked in the muscles of the back and neck, while lesions of nerve sheaths were not found remote from the hemorrhagic areas in the muscles. The cultures of emulsions from the hemorrhagic muscles showed *Staphylococcus aureus* in both rabbits, and no streptococci. Cultures of the blood, joint and cerebrospinal fluids, kidneys, spleen and normal muscle were sterile.

On the basis of these findings, tonsillectomy was performed. The extirpated tonsils contained several large abscesses. Following the removal of the tonsils the fever and the pain in the muscles of the back largely disappeared. The nervousness and lack of endurance persisted for some months, but after the administration of thyroxin, on the advice of Dr H S Plummer, improvement in these symptoms occurred.

DESCRIPTION OF SYMPTOMS AND LESIONS IN ANIMALS

The animals injected with streptococci from the patients with generalized myositis usually developed numerous widely disseminated lesions, while those injected with strains from patients with localized areas of myositis usually developed only localized lesions in the muscles. The former often presented quite characteristic symptoms from which the existence of myositis was frequently predicted. The muscles appeared tender to touch. The animals sat around quietly, with ruffled fur, sometimes resting the nose on the floor of the cage. They were disinclined to move and when made to do so, walked about as if muscular effort

caused pain and discomfort. They lost markedly in weight and often became very weak. The rabbits showing only localized lesions were usually free from demonstrable symptoms, and lost little or no weight. In some instances in both groups of animals, however, the discomfort from muscular effort noted during life could not be explained satisfactorily by the few gross lesions which were found in the muscles after death. In these animals, lesions of nerves, or hemorrhagic areas of muscles or fascia surrounding nerves were often demonstrable (Figs 2, I, III, 3, I, II).

The lesions were of two main types. One type consisted of numerous small linear or oblong hemorrhages (Fig 1, I), the other consisted of equally numerous small linear, grayish white, necrotic streaks (Fig 1, II). The former was, in general, the early stage of the latter, and both types were often present in the same animal in varying proportions. The time when the hemorrhagic lesions became necrotic varied greatly, depending to some extent on the size of the dose injected, but most of all on the strain of streptococcus. Following the injection of some strains, only necrotic areas were found, even as early as twenty-four hours after injection, with other strains, only hemorrhagic areas were found, and these often disappeared without becoming necrotic. The lesions obtained with staphylococci were quite different. Suppuration frequently occurred, and the surrounding edema and hyperemia were more pronounced. The small hemorrhagic and necrotic lesions due to streptococci were often widely distributed throughout the skeletal muscles. They were nearly always more numerous in the flat muscles of the trunk, along the tendinous partitions, and in the tendinous ends of the muscles of the extremities. This picture was in sharp contrast to that following the injection of the strains from cases of localized myositis. Here disseminated lesions rarely developed, but instead localized areas of large and small hemorrhages, associated early with little or no edema and infiltration, while later the edema, infiltration and necrosis often became marked. Softening, with pus formation, was rare, but it occurred more often in the animals with large localized lesions than in those with small, widely disseminated lesions.

The microscopic findings were very interesting. The lesions usually developed between the muscle fibers, and consisted, first, of extravasation of red blood corpuscles, dilatation of adjacent capillaries, and swelling of the muscle fibers with disappearance of the transverse striations as interstitial edema and leukocytic infiltration became manifest. Later, in the larger lesions, the muscle fibers became fragmented and necrotic as leukocytes and other cells became numerous. The amount of cellular infiltration varied from large areas of infiltration (Fig 6, I) to the aggregation of only a few cells between the muscle fibers (Fig 6, II). Deposits of calcium salts occurred relatively early

in the necrotic areas (Fig 7, II), especially in the rabbits after the injection of the streptococcus isolated from the muscle in the case of calcifying myositis (Fig 7, I). A marked deposit of calcium was noted as early as forty-eight hours after the injection of this strain, whereas, after the injection of other strains when necrotic lesions were fewer, little or no calcification was noted. Areas of necrosis of considerable size appeared necessary for the deposit of calcium, a fact which seems to support Wells' theory of calcification¹⁷. There was usually no difficulty in demonstrating the organism in the lesions soon after injection (Figs 8, II, 9, III, 10, III, IV), but later this was often difficult and sometimes impossible. The microscopic changes in the human muscles in acute myositis (Fig 4, I, II) were similar to those produced experimentally. They consisted of extravasation of red blood corpuscles, interstitial edema, cellular infiltration, swelling and necrosis of the muscle fibers, with disappearance of transverse striations. The demonstration of streptococci was also possible (Fig 10, I, II). The whole picture parallels quite closely the description by Kormoczi¹⁸ of the microscopic findings in muscles in a patient who died of acute myositis caused by a streptococcus.

The sections of atrophic and tender muscles and periarticular structures in chronic myositis and arthritis showed relatively little cellular infiltration (Fig 5, I), but showed a marked swelling of the endothelium and sometimes complete plugging of the blood vessels from proliferation of endothelial cells (Fig 5, I, II, III, IV). Streptococci have been isolated by cultural methods from these excised tissues and demonstrated in the endothelial cells, but not without prolonged search.

Owing to these findings it was thought that a study of the changes in the blood vessels in the muscles of rabbits showing lesions might throw some light on the mechanism involved in the localization of the bacteria and the production of the lesions. The changes in the small blood vessels were found to consist of two quite distinct types, aggregation of leukocytes within the lumen (Fig 8, I), and more rarely in the perivascular lymph spaces. This occurred commonly after the injection of strains from patients with acute myositis, who recover promptly. The demonstration of bacteria in the wall of these vessels was not accomplished, but in a few instances diplococci were found within the leukocytes. The small vessels following injection of the strains from chronic generalized myositis, especially after repeated injections of the dead organisms, presented a very different picture. There were few or no leukocytes even soon after injection. The reaction

17 Wells, H. G. Text Book of Chemical Pathology, Philadelphia W. B. Saunders Co., 1918, pp. 707.

18 Kormoczi, E. v. Durch Streptokokkeninfektion verursachte Polymyositis (Polymyositis Streptomycotica), *Centralbl. f. Bakteriologie* **31** 688, 1902.

appeared to be chiefly endothelial. The endothelial cells lining the vessels were often markedly swollen (Fig 8, III, VI), and diplococci were sometimes demonstrable (Fig 8, II). Proliferation of endothelial cells containing deeply staining nuclei occurred in some arterioles, especially in prolonged experiments where repeated injections of liquor formaldehydi-killed streptococci were given (Fig 9, I). The changes noted in the small blood vessels in response to the intravenous injection of bacteria recall the peculiar behavior of small blood vessels noted by Hooker¹⁹ in the ear of the cat during life following the injection of hystamin and other substances.

During the microscopic study, attention became directed to what, at first, were thought to be cross sections of blood vessels in which the lumen was filled with endothelial cells, but which on closer study were considered to be peripheral sensory end organs (Fig 9, II). The cells of these differed from swollen endothelial cells in being more nearly round, and in that nuclei were usually situated to one side. After prolonged search undoubted diplococci were found in these highly specialized nerve cells (Fig 9, III).

LOCALIZATION OF DEAD STREPTOCOCCI FROM MYOSITIS

It was thought that the injection of the dead bacteria as a control might throw light on the mechanism of the production of myositis. Particularly suitable cultures, those in which the living organisms produced marked lesions in muscles, were used. Liquor formaldehyd was selected as the most desirable agent with which to kill the streptococci. One of us had found previously that liquor formaldehyd-killed pneumococci failed to autolyze. The agglutinability of typhoid bacilli killed with liquor formaldehydi remains unchanged for a long time.

After growing the streptococci in tall columns of ascites-glucose broth for from eighteen to twenty-four hours, there were added 0.5 cc liquor formaldehydi (40 per cent strength) for each 100 cc of the culture. This was thoroughly mixed and allowed to stand at room temperature for twenty-four hours, when sterility tests were made. The dead bacteria were then removed by centrifugation, and the sediment, which was drained dry, was suspended in 0.85 per cent salt solution so that 1 cc contained the growth from 15 cc of the broth culture. From 0.5 to 10 cc of this suspension, representing the growth of from 7.5 to 150 cc of broth, were injected into rabbits weighing from 1,050 to 2,150 gm. In most of these animals repeated daily injections, as high as six, were given. The animals were sacrificed usually in from one to ten days after the last injection. The effect of dead streptococci from five cases of myositis (Cases 130, 369, 567, 601, and 904) was studied in eleven rabbits. Lesions similar in location and distribution to those obtained with the corresponding live bacteria were obtained in eight animals following injection of the strains from four cases. No lesions were found in the other three, all of which were injected with small doses.

In the case of dental neuritis and cervical myositis (Case 567) the three rabbits had lesions in the flat and tendinous partitions of muscles and dental pulps. One of these was injected once with the dead streptococci from the

19 Hooker D. R. The Functional Activity of the Capillaries and Venules, *Am J Physiol* 54 30, 1920

primary growth of the excised muscle, one on four occasions with this strain in the second culture from a single colony on blood-agar, and one with the dead streptococci from the primary culture from the infected tooth pulp. The Berkefeld filtrate of the supernatant broth was injected intravenously daily into one rabbit for three days in 12 cc doses. It remained well and was anesthetized for examination on the fourth day. No lesions were found. The lesions following the injection of the dead organism were less numerous and smaller than those following the injection with the corresponding live culture, even though the dose of the dead bacteria was much larger. The lesions consisted of small hemorrhages with little edema and infiltration. In some areas dilatation of blood vessels was found microscopically, in others, constriction, with swelling of endothelial cells. In some instances, the vessels were partially or completely filled with endothelial cells. Intravascular and interstitial leukocytic reactions were slight or absent. The changes in the muscle fibers consisted chiefly of swelling, disappearance of the striations, occasionally of fragmentation, and at times of beginning fatty degeneration.

The demonstration of the streptococci in the tissues was difficult. Most of the bacterial cells were disintegrating and had lost much of the gram stain. Unmistakable diplococci, sometimes in short chains, were found within swollen endothelial cells lining the small blood vessels in the areas of hemorrhage (Fig 8, II), between the muscle fibers, and within what appeared to be sensory end organs (Fig 9, III).

DISCUSSION AND SUMMARY

Localized infections around teeth and in tonsils were demonstrably present in nearly all of the twenty-eight patients studied. Improvement in symptoms, often striking, occurred in all but one of the twenty-five patients from whom foci were removed. In the twenty-four patients in whom improvement did occur the focus was shown to contain bacteria which tended to produce lesions in the muscles of animals, while in the one patient in whom improvement did not occur the bacteria failed to produce lesions. In some patients recurrence of myositis or only partial recovery was found to be due to defective tonsillectomies, to inadequate dental operations, to failure in recognizing the existence of foci in teeth, or to the development of new localized areas of infection. Therefore, localized infections in tonsils, teeth and elsewhere, in the patients studied appeared to play an important part not only in the etiology of pure myositis, but also in associated conditions, such as myositis and arthritis, myositis and neuritis, nontraumatic lumbago and myocardial degeneration. In the light of these facts, foci of infection apparently not only make a forced relationship between the bacteria and their products and the mechanism of resistance of the host, but, it would seem, afford the conditions favorable for the microorganism to acquire peculiar infecting powers.

The elective affinity of the streptococci from these localized infections was so marked, that the organisms not only tended to localize and produce lesions in muscles, but the location of the lesions often approximated that noted in the patient. When the affection of other structures, such as joints and nerves, appeared as a secondary factor in the patient's symptomatology, lesions were also found in these struc-

tures in the injected animals, and the percentage of animals showing them was proportionately less than the percentage of animals showing muscle lesions. In pregnant rabbits localization was found to occur not only in the muscles of the parent rabbit, but also in the fetuses.

The localization in the myocardium, especially in the bundle of His, and the concomitant development of arrhythmia of the heart following the injection of cultures from pyorrheal pockets, in the case of impaired myocardial conduction, is another striking example of the extreme specificity of these strains for certain tissues. This narrow specificity for certain tissues is in keeping with the fact that myositis occurs during certain epidemics of respiratory infections, and with the striking example of elective localization in a wide-spread epidemic of myositis reported by Curschmann,²⁰ in which the muscles of the neck were chiefly involved.

The streptococcus from myositis did not differ greatly in morphology, cultural character, and staining reactions from those isolated in other diseases studied, and the reasons for the myositis strains possessing affinity for muscles, and the other strains for the respective other tissues or organs are still obscure. The fact, however, that the killed streptococci from myositis localized in muscles, as did the live organism, indicates that the property (chemical structure, electrical charge, perhaps) on which this depends resides within the bacterial cell. The observation that the dead streptococci revealed evidence of disintegration as lesions in the muscles developed suggests that the poisons which cause the symptoms and lesions in myositis are either preformed in the bacterial cells or are formed as they disintegrate. The latter view is strongly supported by the experiments performed by one of us²¹ some years ago, in which it was shown that when streptococci and other bacteria undergo digestion highly poisonous substances of a similar nature, regardless of the species, are formed during the early stages of proteolysis and that these disappear as proteolysis proceeds to a further point. According to the results of these experiments and those of Zinsser²² and his coworkers, in which toxic substances are formed similar in their effects, irrespective of the species of micro-organism, the localizing power of bacteria rather than specific poisons becomes a factor of first importance in the etiology of myositis, as indeed it may be of other diseases.

20 Curschmann, H. Ueber eine Epidemie von myositischer Pseudogenickstarre, *Munchen med Wchnschr* **44** 1, 1917, Abstr. *J. A. M. A.* **68** 943, 1917.

21 Rosenow, E. C. On the Production of Anaphylatoxic Substances by Autolysis of Bacteria and Their Relation to Endotoxins, *J. Infect. Dis.* **10** 113, 1912.

22 Zinsser, H., Parker, J. T. and Kuttner, A. On Certain Poisonous Substances Produced in Bacterial Cultures, *Proc. Soc. Exper. Biol. and Med.* **18** 49, 1920.

The retention of the peculiar affinity during the killing process of these streptococci is in keeping with the fact discovered by Marchand,²³ that opposition of virulent organisms to phagocytosis is not destroyed by heat, and to the observation by one of us²⁴ that the resistance to phagocytosis of virulent pneumococci is due to an extractable substance, "virulin." The fact that the streptococci having affinity for the muscles in these cases are culturally dissimilar does not argue against their having etiologic relationship. The symptoms and the course of the disease are so variable as to suggest their not being due to a specific micro-organism. The streptococci from the joints of patients with acute rheumatic fever, having elective affinity, it will be recalled, were also dissimilar.⁸

The finding of the *Staphylococcus aureus* in the tonsils in Case 536 having elective affinity for muscles indicates that this organism may be the cause of mild myositis, just as Martinotti,²⁵ and Schmitz²⁶ have shown this to be so in one case each of acute polymyositis, in which this organism was isolated from the muscle lesion and was found to have elective affinity for the muscles of animals, producing lesions in muscles almost to the exclusion of lesions in other organs.

From the microscopic study of sections of muscles in chronic myositis and arthritis deformans, and of those in animals following repeated injections of these strains, a most interesting mechanism in the production of the lesions has been discovered. Evidence has been obtained as to why cure in these chronic affections is so difficult, and the reason massage and applications of heat are such valuable agents in their treatment. The reaction in these very chronic conditions is not leukocytic, but mainly mononuclear and endothelial. The endothelial cells lining the small blood vessels become extremely swollen, proliferate, and, in consequence, the lumen of vessels, including arterioles, becomes partially or completely obstructed. The supply of available oxygen is thus lowered and the growth of the organisms favored, for it has been found that the streptococci from the lesions in muscles are very sensitive to oxygen. The common occurrence of spontaneous and experimental lesions in flat muscles or in the tendinous ends where the normal blood supply, and, consequently, the supply of available oxygen in response to insult are relatively low would seem to be due to the same cause.

23 Marchand, L. Etude sur la phagocytose des streptocoques atténues et virulents, Arch de med exper **10** 253, 1898

24 Rosenow, E. C. Human Pneumococcal Opsonin and the Anti-Opsonic Substance in Virulent Pneumococci, J Infect Dis **4** 285, 1907

25 Martinotti, C. Ueber Polymyositis acuta verursacht durch einen Staphylococcus, Centralbl f Bakteriöl **23** 877, 1898

26 Schmitz, H. Bakteriologische Untersuchung eines Falles von Polymyositis acuta, Centralbl f Bakteriöl, Orig **65** 259, 1912

Our success in isolating the strains and in reproducing the lesions is due, we feel, in large part to the use of culture mediums (tall columns of glucose-brain broth or ascites broth) which afford a gradient of oxygen pressure, to the injection of animals as soon as abundant growth occurred, usually within twenty-four hours, and to the painstaking effort made to obtain bacteria from the depths of foci of infection

Bacteria having specific affinity for muscles have been demonstrated regularly in the foci of infection and, in some instances, on the free surface of mucous membrane and in excised muscles in cases of myositis in man. With these organisms the disease has been reproduced, the organisms isolated from the experimental lesions, and demonstrated in them, and myositis again produced on reinjection. The lesions in animals, in general, corresponded in their severity and distribution to those present in the patient from whom the strains were isolated, and in that they were usually nonsuppurative in character. The number of lesions was often in proportion to the size of the dose. The conclusion, therefore, may be drawn that myositis, including even the mild transient affections of muscles, is caused in the main by lodgment and growth of bacteria, usually streptococci, which have elective affinity for muscle tissue²⁷

²⁷ Evans, J. S. Focal Infections. In Tice, F. Practice of Medicine, Hagerstown, Md., W. F. Prior Co., 1920, 1

ON THE CAUSES OF THE VARIATIONS IN THE SEDIMENTATION OF THE CORPUSCLES AND THE FORMATION OF THE CRUSTA PHLOGISTICA ("SIZE," "BUFFY COAT") ON THE BLOOD

A PRELIMINARY COMMUNICATION *

H C GRAM
COPENHAGEN, DENMARK

If venous blood from a normal man or woman is left to clot it will, as a rule, form a homogenous red mass at the end of the coagulation

In certain pathologic and physiologic states, the coagulum is differentiated into a lower red and an upper, more or less broad, yellowish layer (crusta phlogistica, buffy coat, size), which consists of coagulated plasma

While this phenomenon is normal in the blood of several animals, it has been known from the earliest times that a crusta phlogistica in human blood is a pathologic phenomenon

The newer medicine, however, generally has disregarded this symptom, possibly because it may be found in so many different states, which did not seem to form a unity This opinion, for instance, is thus expressed by Panum¹ (1851) "*Utrum crusta in sanguine appareat, necne, ex rationibus compluribus vere physicis pendet, et vix unquam licet inde certam sanguinis compositionem suspicari*"

Investigations by Hewson² (1771) and Hunter³ (1794) had proved that a buffy coat on the blood appeared when the sedimentation of the corpuscles or the time of coagulation was increased Both authors put stress on the changes of the clotting time

A crusta phlogistica was found especially in cases of inflammation, so that Borsieri⁴ and Tommasini,⁵ who found this phenomenon in chlorosis, thought that this disease might be characterized as an inflammation

Panum,¹ however, warned against repeated venesection in patients because their blood showed a crusta phlogistica, since this might be caused by the bloodletting itself

Andral⁶ (1845) studied the conditions under which the crusta (couenne) formation took place clinically, and came to the conclusion

* From the Clinic of Professor Faber, Rigshospital

1 Panum Dissertation (Copenhagen) 1851

2 Hewson Experimental Inquiries in the Properties of Blood, London, 1771

3 Hunter Treatise on the Blood, London, 1794

4 Borsieri Loco incerto, quoted by Andral⁶

5 Tommasini Sull' infiammazione quoted by Andral⁶

6 Andral Essai d'hematologie pathologique, Paris, 1845

that a crusta on the blood appeared only when the fibrin percentage of the blood was increased or the number of corpuscles was decreased

Henle,⁷ Dogiel,⁸ Panum,¹ Hayem,⁹ Valentin¹⁰ and Pfeiffer¹¹ all found that the sedimentation of the corpuscles was of extreme importance for the formation of the buffy coat, and that an increased sedimentation of the corpuscles generally was caused by an agglutination of the corpuscles

Pfeiffer thought that an increased fibrinogen percentage might make the corpuscles sticky and thus further the agglutination

Hekma¹² showed that the corpuscles of the horse sank more rapidly in citrated blood than in defibrinated blood, and de Haan¹³ found that the velocity of sedimentation was proportional with the degree of agglutination. The cause of the agglutination according to de Haan lies in the corpuscles, though he admitted that the medium of suspension had some influence

Kozawa¹⁴ and Høber¹⁵ showed that there was a parallelism between the negative electrical charge of the corpuscles in different animals and the differences between the rapidity of sedimentation found by Berkzeller and Stanker¹⁶

Schwytzer¹⁷ has investigated these relations more fully and finds that the suspension stability of the corpuscles depends on the negative charge of the corpuscles

When this decreases, the corpuscles are attracted to one another and agglutinate. While citrate did not hamper the agglutination, a surplus of sodium chlorid (11 *pro mille*) prevented it

Fåhræus¹⁸ specially studied the augmented sedimentation found during normal pregnancy. He found a proportionality between the sedimentation velocity and the degree of auto-agglutination

The phenomenon, however, was not specific, since it was found also in most infectious diseases and in cases of cancer. Also the sedimentation was quicker in the blood of normal women than in that of normal men

In a later paper Fåhræus¹⁹ discusses the causes of the changes in the stability of the blood, accepting the theory of the lowered electrical

7 Henle Loco incerto

8 Dogiel Arch f Anat u Physiol, 1883, quoted by Pfeiffer

9 Hayem Du sang, etc, Paris

10 Valentin Pathologie des Blutes, Leipzig, 1866

11 Pfeiffer Ztschr f klin Med **33** 215, 1897

12 Hekma Biochem Ztschr, Festband, p 177, 1908

13 de Haan Biochem Ztschr **86** 298, 1918

14 Kozawa Biochem Ztschr **60** 146, 1914

15 Høber Arch f d ges Physiol **101** 627, 1904

16 Berkzeller and Stanker Internat Ztschr f physiol chem Biol **3** 133, 1917

17 Schwytzer Biochem Ztschr **60** 297, 1914

18 Fåhræus Hygiea, 1918

19 Fåhræus Biochem Ztschr **89** 115, 1918

charge as the reason for the augmented agglutination. He finds that the properties of the corpuscles are altered, but that the primary reason for this change is to be sought in the plasma.

As to the provocative cause, Fåhræus remarks that the sedimentation is increased in all cases in which Abderhalden supposes that proteids foreign to the blood are introduced into the circulation.

Quite recently Westergren²⁰ published a paper dealing with the rapid sedimentation found in cases of pulmonary tuberculosis. In this disease he constantly finds an increased sedimentation, beyond the normal values, when citrated blood is kept in narrow tubes and observed after one hour.

In this paper there is mentioned an as yet unpublished research by Fåhræus on the causes of the sedimentation.

While working on the causes of hemorrhagic diathesis, I have elaborated a technic,²¹ by which the fibrin percentage in blood and plasma may be determined by a gravimetric method on 2 c c cell-free citrated plasma obtained from 5 c c citrated blood.

The blood (4.5 c c) was taken into a graduated centrifuge tube (Oluf Thomsen) containing 0.5 c c of a 3 per cent citrate solution. In many cases the platelet count (Thomsen,²² Gram²³) was determined by Thomsen's method and also the coagulation time of citrated plasma on recalcination (Gram²⁴). The tubes used were divided into 0.1 c c, and the size chosen was such that 0.1 c c on the gradations equals 0.1 cm.

In order to calculate the platelet count and fibrin percentages, the cell volume was determined in all cases.

I soon found that there occurred a more or less pronounced sedimentation in these blood specimens, and that the sedimentation depended on (a) the cell volume percentage, and (b) the fibrin (fibrinogen) percentage in the plasma.

The sedimentation ordinarily was measured by observing the height in centimeters of the plasma layer formed, when the 5 c c citrated blood was left to stand at room temperature (about 19 C) for ten minutes. The plasma layer was measured between the lower meniscus of the plasma surface and the red cell layer. Excepting in anemias, this borderline was nearly always quite clear and sharp as if cut with a knife.

20 Westergren. Acta med scand **54** 247, 1921.

21 The technic for the fibrin determinations will be published later, together with the results in each separate case.

22 Thomsen. Acta med scand **53** 507, 1920.

23 Gram. Arch Int Med **25** 325 (March) 1920, Acta med scand **54** 1, 1920.

24 Gram. Bull Johns Hopkins Hosp **31** 364, 1920 (see corrections in the following issues).

In some ways it is most convenient to use an observation time of only ten minutes, but one must remember that cases with very different, generally normal fibrin percentages and cell volumes are thus included in the same group showing less than 0.1 cm (1 c traces) of sedimentation. However, in many pathologic cases the sedimentation after one hour has progressed as far as it will go, thus making the differences in these cases smaller than by an observation after ten minutes.

The formation of the crista phlogistica was observed by letting 1 cm³ of blood flow down in a miniature test tube having a diameter of from 9 to 10 mm, leaving it to clot in an upright position of the glass. The reaction could not be measured exactly, since the border between crista and corpuscles generally is not very sharp. I have distinguished between — (no crista), traces, + (positive) and ++ (very broad).

The result of the cell volume determinations has been, that the cell volume in normal women oscillates about an average of 41 per cent of the pure blood and between the limits 37 and 45 per cent.

In normal men, the cell volume oscillates about 48 per cent, and between the extremes 43 and 51 per cent.

A low cell volume, of course, was found in simple anemias, pernicious anemia and most cases of leukemia, pseudoleukemia and several other diseases of the blood. Some infectious diseases, as, for instance, rheumatic fever, typhoid, erysipelas and chronic tuberculosis, very often showed a low cell volume.

In cases of nephritis, and in cases of polyarthritis chronica and malignant tumors one may find a low cell volume. The same is true in normal pregnancy, which, especially in the earlier months, shows a distinct drop in the cell volume percentages.

The fibrin percentage per 100 cm³ plasma—which is the value to be considered—in twenty-five normal individuals of each sex varied between 0.20 and 0.38 per cent, the mean values being 0.27 per cent in men and 0.29 per cent in women, that is, slightly lower in men than in women. These different levels of fibrin content are held from one time to another by the same normal individual.

In many chronic noninfectious diseases no deviations were found from the normal fibrin content of the plasma and the normal cell volume.

In the description of each separate disease, the number of cases observed and the average and variations of the fibrin percentage in plasma is added in brackets.

A fibrin percentage in plasma below the lowest normal limit was met with in some but not nearly in all cases of severe degeneration of the liver.

Out of fourteen such cases, six showed a fibrin percentage in plasma smaller than 0.20 per cent, and the average was 0.22 per cent (variations from 0.39 to 0.07 per cent). In some of these cases, when infectious complications were present, it appears that the normal fibrin percentage found must be considered pathologic. The diagnosis in all cases was verified postmortem.

Outside this group a fibrin deficiency was found only in a few cases of pernicious anemia²⁵ (fatty degeneration of the liver?) and one case of profuse carcinomatosis of the liver.

In simple anemia (fifteen cases, average 0.33 per cent, variations from 0.40 to 0.25 per cent) the fibrin percentage in plasma was normal, though the percentage per 100 c c blood was very high.

In polycythemia (eight cases, average 0.30 per cent, variations from 0.40 to 0.21 per cent) the percentage in plasma also varied nearly within the normal limits, while the percentage per 100 c c blood was very low. This means that the fibrin percentage in plasma, not that in blood, is the value which remains fairly constant, when the cell volume is altered.

In leukemia (fourteen cases, average 0.37 per cent, variations from 0.46 to 0.29 per cent), especially in the myeloid type, one may find a slight absolute increase of the fibrin percentage in the plasma. The same is true in hemophilia (three cases, average 0.40 per cent, variations from 0.43 to 0.37 per cent).

One case of purpura and one of pseudoleukemia showed normal fibrin percentages (respectively 0.31 and 0.34 per cent), while a case of scurvy with gingivitis and loosened teeth had a slight increase (0.41 per cent).

The increase is very pronounced and always absolute (i. e., > 0.38 per cent) in lobar pneumonia (seven cases, average 1.05 per cent, variations from 1.50 to 0.80 per cent), bronchopneumonia (five cases, average 0.75 per cent, variations from 1.29 to 0.52 per cent), rheumatic fever (nine cases, average 0.77 per cent, variations from 0.94 to 0.55 per cent), pleurisy (eight cases, average 0.74 per cent, variations from 1.07 to 0.55 per cent), erysipelas (four cases, average 0.72 per cent, variations from 0.87 to 0.58 per cent), suppurative infections (sixteen cases, average 0.68 per cent, variations from 1.01 to 0.46 per cent), diphtheria (five cases, average 0.63 per cent, variations from 0.73 to 0.48 per cent), gonorrheal "metastasis" (four cases, average 0.61 per cent, variations from 0.81 to 0.48 per cent), scarlet fever (sixteen cases, average 0.60 per cent, variations from 0.83 to 0.45 per cent), angina (ten cases, average 0.57 per cent, variations from 0.80

²⁵ Pernicious anemia. 23 cases, average 0.26 per cent, variations from 0.37 to 0.10 per cent. Only four cases show less than 0.20 per cent fibrin in the plasma.

to 0.46 per cent) and bronchitis (fifteen cases, average 0.57 per cent, variations from 0.91 to 0.40 per cent)

The increase is slighter and may keep within the upper boundaries of the normal in uncomplicated measles (two cases, average 0.42 per cent, variations from 0.42 to 0.43 per cent), typhoid (five cases, average 0.44 per cent, variations from 0.50 to 0.36 per cent), influenza (thirty-three cases, average 0.38 per cent, variations from 0.48 to 0.30 per cent), "closed tuberculosis" (seven cases, average 0.36 per cent, variations from 0.43 to 0.25 per cent), malaria (three cases, average 0.35 per cent, variations from 0.38 to 0.30 per cent), and syphilis (six fresh cases, average 0.35 per cent, variations from 0.42 to 0.27 per cent and six old cases, average 0.47 per cent, variations from 0.66 to 0.31 per cent)

If one of these last named infections is complicated by one belonging to the first group, there occurs an immediate rise in the fibrin percentage

This was demonstrated in (1) measles with bronchitis or otitis (four cases, average 0.53 per cent, variations from 0.58 to 0.47 per cent), (2) influenza with bronchitis (five cases, average 0.55 per cent, variations from 0.66 to 0.48 per cent), (3) influenza with angina or otitis (three cases, average 0.58 per cent, variations from 0.60 to 0.57 per cent), (4) influenzal pneumonia (twenty-two cases, average 0.62 per cent, variations from 0.98 to 0.36 per cent), (5) tuberculosis of the lungs (twenty-one cases, average 0.62 per cent, variations from 1.22 to 0.33 per cent)

Outside of the infectious diseases proper, an increased fibrin percentage in the plasma was often or always found in the following pathologic and physiologic states (1) After intramuscular injection of sterile milk (two cases, average 0.465 per cent, variations from 0.48 to 0.45 per cent), (2) polyarthritis chronica progressiva (ten cases, average 0.525 per cent, variations from 0.70 to 0.36 per cent), (3) malignant tumors (excluding cancer of the liver or pancreas twenty-six cases, average 0.55 per cent, variations from 0.81 to 0.31 per cent), (4) nephritis (thirteen uncomplicated cases, average 0.41 per cent, variations from 0.80 to 0.27 per cent, ten cases with fever or lung complications, average 0.62 per cent, variations from 0.89 to 0.50 per cent), (5) pregnancy (second month, one case, average 0.33 per cent, third month, one case, average 0.42 per cent, fourth month, three cases, average 0.36 per cent, variations from 0.39 to 0.33 per cent, fifth month, four cases, average 0.39 per cent, variations from 0.44 to 0.32 per cent, sixth month, nine cases, average 0.43 per cent, variations from 0.49 to 0.32 per cent, seventh month, ten cases, average 0.46 per cent, variations from 0.52 to 0.40 per cent, eighth month, seven cases, average 0.57 per cent, variations from 0.83 to 0.44 per cent ninth

TABLE 1—SEDIMENTATION AFTER TEN MINUTES (ABSCISSA), CELL-VOLUME PERCENTAGE (ORDINATE) AND FIBRIN PERCENTAGE IN PLASMA (RUBRIC NUMERALS) IN FIVE HUNDRED AND FORTY-TWO BLOOD SPECIMENS THE RUBRIC NUMERALS ARE MEAN VALUES OF THE SPECIMENS, WHICH SHOW AN EQUAL SEDIMENTATION AND A NEARLY EQUAL CELL-VOLUME*

		Sedimentation after 10 Minutes in Centimeters																									
0	Trace	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6
0-10																					0.42						
11-15		0.10	0.26	0.32	0.32	0.32			0.39	0.32	0.33		0.44														
16-20			0.23	0.26	0.28	0.28	0.31		0.35		0.27		0.30														
21-25	0.20	0.25	0.23	0.30	0.31	0.40	0.38		0.46			0.58	0.72			0.61	0.59	0.63									
26-30	0.10	0.27	0.24	0.27	0.32	0.37	0.43	0.48		0.33					0.72	0.69				0.78						0.92	
31-35		0.29	0.38	0.47	0.52	0.65	0.56	0.56		0.71		0.94	0.70		0.70	0.78	0.94						0.98				
36-40		0.33	0.47	0.51	0.53	0.59	0.79	0.78	1.02	0.82		0.75	0.71	0.97	1.22	0.85	0.83										
41-45	0.24							0.86																			
46-50	0.31	0.52	0.59	0.58	0.55	0.51	0.70																				
51-55	0.35	0.19				0.73																					
56-60	0.25																										
61-65	0.31																										
66-70	0.28																										
	0.30																										

* In considering this table one must remember that a sedimentation <0.1 cm after ten minutes may be met with in specimens with a normal cell volume, but a slightly increased fibrin percentage. In severe anemia the borderline between plasma and corpuscles is not clearly defined, so that the reading must be somewhat arbitrary.

month, seven cases, average 0.49 per cent, variations from 0.56 to 0.45 per cent, tenth month, eighteen cases, average 0.55 per cent, variations from 0.74 to 0.39 per cent), (6) catarrhal jaundice (four cases, average 0.38 per cent, variations from 0.54 to 0.29 per cent), (7) cholelithiasis (two cases, average 0.415 per cent, variations from 0.42 to 0.41 per cent)

These "hyperinoses" may in some cases be explained by complicating infections

This holds true in connection with the increased fibrin percentages found in many cases of decompensated heart disease and some cases of nephritis with stasis or edema of the lungs. Sometimes, however, the stasis in the liver counteracts the influence of the infection in the lungs, making the fibrin percentage normal or even decreased

TABLE 2—INFLUENCE OF VARIATIONS IN FIBRIN PERCENTAGE AND CELL VOLUME

Diagnosis	Date	Sedimentation in 10 Minutes, Cm	Crusta Phlogistica	Fibrin in Plasma, per Cent	Cell-Volume, per Cent
1 Simple anemia	1/ 6	0.2	+	0.32	21
	1/17	0.2	Traces	0.29	21
	2/10	0.1	+	0.32	21
	2/24	0.1	+	0.29	25.5
	3/ 9	0.1	—	0.26	33
	3/23	Traces	—	0.30	39
	3/30	Traces	—	0.31	41
2 Rheumatic fever, anemia	2/ 1	1.6	++	0.94	34
	2/14	0.3	—	0.45	34
	2/21	0.2	+	0.43	37
	2/28	Traces	—	0.35	35.5
	3/ 6	0.1	—	0.32	38

The hyperinosis found in cases of malignant tumors may partly be explained by infection of ulcerated surfaces, and, in fact, one case with a closed, small cancer in the vertebral column showed no absolute increase. Still, there can be no doubt that the tumor itself exerts some influence in the same direction.

Icterus per stasis in cases of cancer of the pancreas does not prevent the hyperinosis (four cases, average 0.63 per cent, variations from 0.97 to 0.50 per cent). In secondary, diffuse cancer of the liver the fibrin percentage in plasma may rather often, especially in the last stages, be found normal or even decreased (seven cases, average 0.47 per cent, variations from 0.72 to 0.27 per cent [first examinations!])

From the beginning of normal pregnancy²⁶ there occurs a tendency to hyperinosis, which however does not always pass beyond the upper boundary of normal before in the fifth or sixth month

26 At the same time there is a more or less pronounced tendency to anemia

There is no special increase in cases of eclampsia, in which, however, the cell volume is markedly higher than in normal pregnant women (three cases, average 0.57 per cent, variations from 0.70 to 0.48 per cent)

The sedimentation of the corpuscles was studied on 542 specimens of citrated blood

The result of this series (Table 1) was that a sedimentation of 0.1 cm or more after ten minutes occurred only (1) when the fibrin percentage in plasma was higher than normal, or (2) when the cell volume was lower than normal

The effect of variations in these factors is shown in Table 2

In order to demonstrate the effects of the finer changes in the fibrin percentage, a longer time of observation is necessary, but this has only been done in a few cases, which, however, showed that the sedimentation in normal blood was regulated by the same laws

TABLE 3 — CELL VOLUME, FIBRIN PERCENTAGE AND SEDIMENTATION AFTER ONE HOUR IN TEN INDIVIDUALS OF EACH SEX

Number	Cell Volume per Cent	Fibrin Percentage in Plasma	Sedimentation after 1 Hour	Number	Cell-Volume, per Cent	Fibrin Percentage in Plasma	Sedimentation after 1 Hour
1	38	0.37	1.3	1	49	0.37	0.5
2	40	0.33	0.8	2	49	0.35	0.4
3	38	0.29	0.6	3	46	0.33	0.3
4	43	0.31	0.6	4	49	0.31	0.1
5	45	0.35	0.5	5	46	0.24	0.1
6	40	0.26	0.5	6	49	0.27	<0.1
7	40	0.27	0.4	7	48	0.24	<0.1
8	42	0.24	0.4	8	45	0.21	<0.1
9	43	0.27	0.4	9	46	0.225	<0.1
10	46	0.32	0.2	10	49	0.23	<0.1
Average	41.5	0.30	0.6		48	0.27	<0.2

After ten minutes one may observe, that the plasma layer formed is broader in women's than in men's blood, where it may be missing altogether

An observation of citrated blood from ten normal women and the same number of men after one hour has given the results shown in Table 3. Women to the left, men to the right side

From Table 3 it appears that the difference between the sedimentation in the blood of men and women is very pronounced, and that the difference in fibrin percentage will only partly explain this

The question now is, whether the difference in the average cell volume can explain the different sedimentation found in the two sexes

To decide this I made the following experiments. 20 cm³ of citrated blood is taken from a normal woman, and three Oluf Thomsen tubes are each filled with exactly 5 c.c. of this

The two specimens (1 and 2) are left to stand for one hour, and the sedimentation in centimeters is noted

The third specimen (3) is centrifuged for one and one-half hours. The cell volume and the fibrin percentage in plasma is determined.

To Specimen 2 a little of the precipitate from Specimen 3 is added and a corresponding quantity of the plasma is taken off.

Specimens 1 and 2b are then shaken energetically, and left to stand for another hour, the sedimentation again being noted in both.

Both specimens (1 and 2b) are then centrifuged and the cell volume in both is determined.

TABLE 4—SEDIMENTATION AFTER ONE HOUR IN CITRATED BLOOD BEFORE AND AFTER AN ARTIFICIAL INCREASE OF THE CELL VOLUME. TWO CASES

	Sedimentation after 1 Hour	Cell Volume, per Cent	Fibrin Percentage in Plasma
Experiment A			
1	0.9	40	0.34
2	0.9	40	0.34
1b	0.8	40	0.34
2b	0.3	47	0.34
Experiment B			
1	0.4	43	0.27
2	0.4	42	0.27
1b	0.35	42	0.27
2b	<0.1	49	0.27

These two experiments (Table 4) seem to show that the principal cause of the difference in sedimentation between the blood of the sexes must be sought in the different averages of the cell volume.

There can be no doubt that there is a considerable experimental error in the determination of the velocity of sedimentation by this method. However, a series of double specimens and double specimens with variations in the amount of blood added to the citrate gave nearly uniform values.

A more serious error is introduced by the variations in the room temperature. This is shown in Table 5 on two double specimens which are kept respectively at 20 and 28 Celsius.

TABLE 5—SEDIMENTATION IN TWO PATHOLOGIC DOUBLE SPECIMENS KEPT AT DIFFERENT TEMPERATURES *

Sedimentation in Specimen I Kept at 20 Celsius, Cm	Sedimentation in Specimen II Kept at 28 Celsius, Cm
1.5	2.0
0.3	0.5

* The sedimentation increases with the surrounding temperature till 30 Celsius.

It may also be questioned whether it is the number of cells or the cell volume which influences the velocity of sedimentation.

This was decided by determining the sedimentation on two specimens, respectively, from a case of simple and a case of pernicious anemia (Table 6) with the same cell volume and nearly the same fibrin percentage.

In order to demonstrate more directly that the sedimentation depends on the cell volume and the fibrin (fibrinogen) percentage in plasma, I have carried out a series of special experiments

The sedimentation was measured in the same (Oluf Thomsen) glasses and in the same way as previously

In these experiments measured quantities of corpuscles were suspended in various organic and inorganic liquids

These corpuscles and liquids were prepared in the following way

(1) *Washed Corpuscles*—From 5 to 25 cm of blood is defibrinated, the corpuscles are centrifuged down and the serum is pipetted off. The corpuscles then are washed in 0.9 per cent sodium chlorid and thrown down several times. The last centrifuging is specially energetic. The corpuscular mass is made homogenous by shaking.

(2) *Citrated Serum*—Nine parts of venous blood is defibrinated. One then adds one part of a 3 per cent citrate solution, centrifuges and removes the citrated serum.

(3) *Citrated Plasma*—This is prepared by centrifugalization for one and one-half hours of nine parts of blood + one part of a 3 per cent citrate solution. All cellular elements are thrown down.

TABLE 6—THE SEDIMENTATION DEPENDING ON THE CELL VOLUME

Diagnosis	Erythrocytes Millions per MM	Fibrin Percentage in Plasma	Cell Volume per Cent	Sedimentation in 10 Minutes
Anemia simplex	3.03	0.30	24	0.3
Anemia perniosa	1.73	0.29	24	0.4

(4) *Fibrinogen-Free Plasma*—This is prepared by heating citrated plasma to 60 C in ten minutes. The precipitated fibrinogen is thrown down by centrifugalization.

(5) *Pure Fibrinogen*²⁷—This is prepared by mixing equal volumes of citrated plasma and saturated sodium chlorid solution. The precipitated fibrinogen is redissolved in 0.9 per cent sodium chlorid. The process is repeated three or four times. The fibrinogen content of the final solution is determined by adding fresh serum to a few c.c. The solution is dialyzed over night against 0.9 per cent sodium chlorid to free it of its surplus of chlorids which prevent the agglutination.

(6) *Physiological Sodium Chlorid Solution*—0.9 per cent sodium chlorid.

The questions to be solved by this series of experiments are the following:

(1) *Is the rapid sedimentation in pathologic blood specimen due to properties of the corpuscles or the plasma?*

²⁷ The process is rather delicate and possibly newer methods may give better results (Johns Hopkins Hosp. Bull., 1921, loco incerto).

The experiment shown in Table 7 seems to show that the influence of the plasma is prevalent, though the sedimentation is slower and only speeds up later, when normal corpuscles are suspended in pathologic plasma

TABLE 7—WASHED CORPUSCLES FROM A PATIENT WITH TUBERCULOSIS PULMONUM (FIBRIN PERCENTAGE IN PLASMA 0.59) AND FROM A NORMAL INDIVIDUAL CITRATED PLASMA FROM THE SAME PERSONS

Time After Shaking	1.5 Cm ³ Tuberculosis Corpuscles + 3.5 Cm ³ Tuberculosis Plasma	1.5 Cm ³ Normal Corpuscles + 3.5 Cm ³ Tuberculosis Plasma	1.5 Cm ³ Tuberculosis Corpuscles + 3.5 Cm ³ Normal Plasma	1.5 Cm ³ Normal Corpuscles + 3.5 Cm ³ Normal Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
5 min	0.2	Traces	Traces	Traces
10 min	0.8	0.1	0.1	0.1
15 min	1.7	0.3	0.2	0.3
20 min	2.3	1.4	0.4	0.4
25 min	2.8	2.3	0.5	0.6
30 min	3.0	2.6	0.7	0.8

On the other hand, there is no considerable difference in sedimentation when respectively "normal" and "pathologic" corpuscles are suspended in normal plasma

(2) *Is the velocity of sedimentation altered when the corpuscles are suspended in citrated serum instead of citrated plasma from the same individual?*

The experiment described in Table 8 shows that the sedimentation is enormously decreased when citrated serum is used instead of citrated plasma²⁸

TABLE 8—WASHED CORPUSCLES FROM A PATIENT WITH RHEUMATIC FEVER (FIBRIN PERCENTAGE IN PLASMA 0.90), CITRATED PLASMA AND CITRATED SERUM FROM THE SAME PATIENT

Time After Shaking	2 Cm ³ Corpuscles + 3 Cm ³ Citrated Serum	2 Cm ³ Corpuscles + 3 Cm ³ Citrated Plasma	1 Cm ³ Corpuscles + 4 Cm ³ Citrated Serum	1 Cm ³ Corpuscles + 4 Cm ³ Citrated Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min	0	0.2	0.2	2.0
15 min	Traces	0.8	0.3	3.2
20 min	Traces	1.4	0.6	3.5
25 min	0.1	1.9	0.9	3.6
30 min	0.1	2.2	1.2	3.7

The experiment is carried out twice, respectively with 1 and 2 cm³ corpuscles. It also shows that a lowering of the cell volume increases the sedimentation both in plasma and in serum

28 As the platelets and all other cells are thrown down by the preparation of the plasma, their absence in the serum cannot be the cause of the difference between these two mediums of suspension

(3) *Is the sedimentation altered, when the corpuscles are suspended in "fibrinogen free" plasma instead of citrated plasma from the same individual?*

The experiment shown in Table 9 shows that the sedimentation, *ceteris paribus*, is decreased considerably when the corpuscles are suspended in a plasma deprived of its fibrinogen by heat coagulation

TABLE 9—WASHED CORPUSCLES FROM A PATIENT SUFFERING FROM NEPHRITIS (FIBRIN PERCENTAGE IN PLASMA 0.48), CITRATED PLASMA AND "FIBRINOGEN-FREE" PLASMA FROM THE SAME PATIENT

Time After Shaking	2 Cm ³ Corpuscles + 3 Cm ³ Fibrinogen free Plasma	2 Cm ³ Corpuscles + 3 Cm ³ Citrated Plasma	1 Cm ³ Corpuscles + 4 Cm ³ Fibrinogen free Plasma	1 Cm ³ Corpuscles + 4 Cm ³ Citrated Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min	Traces	0.1	0.5	1.0
15 min	0.1	0.3	0.9	1.7
20 min	0.2	0.5	1.3	2.4
25 min	0.2	0.8	1.7	3.0
30 min	0.3	1.0	1.9	3.2

As in the previous experiment, this also shows that a lowering of the cell volume increases the sedimentation both in ordinary and in fibrinogen free plasma

(4) *Is the sedimentation different when corpuscles are suspended respectively in physiologic sodium chlorid solution and in citrated serum?*

TABLE 10—WASHED CORPUSCLES AND CITRATED SERUM FROM THE SAME PERSON WITH PRESUMABLY NORMAL FIBRIN PERCENTAGE PHYSIOLOGIC SODIUM CHLORID SOLUTION*

Time After Shaking	2 Cm ³ Corpuscles + 3 Cm ³ 0.9% NaCl	2 Cm ³ Corpuscles + 3 Cm ³ Citrated Serum	1 Cm ³ Corpuscles + 4 Cm ³ 0.9% NaCl	1 Cm ³ Corpuscles + 4 Cm ³ Citrated Serum
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min	Traces	Traces	Traces	<0.1
15 min	Traces	<0.1	<0.1	0.1
20 min	Traces	<0.1	<0.1	0.1
25 min	Traces	<0.1	<0.1	0.2
30 min	Traces	<0.1	<0.1	0.3
24 hrs	0.5	1.9	2.8	3.6

* In this and the following tables I have distinguished between traces of sedimentation and a sedimentation <0.1, which latter means that the lower meniscus of the plasma (serum) surface is visible

The experiment shown in Table 10 shows that the sedimentation, *ceteris paribus*, is more rapid in citrated serum than in physiologic sodium chlorid solution. This, however, may partly be due to the action of the sodium chlorid. As in the previous experiments, this has been done with different cell volumes, which shows that a lowering of the cell volume increases the sedimentation both in citrated serum and in physiologic sodium chlorid solution

The specimens have been observed for a longer period, since the sedimentation is very slow

(5) *Do the corpuscles settle more quickly in pathologic than in normal citrated serum?*

The experiment shown in Table 11 tends to show that the corpuscles settle a little more quickly in citrated serum from cases with hyperinosis than serum from normal individuals

(6) *Will the addition of fibrinogen to a liquid cause a more rapid sedimentation of the corpuscles suspended therein?*

The addition of "pure" fibrinogen to a 0.9 per cent sodium chloride solution at first caused a distinct decrease in the sedimentation. The fibrinogen contained a large amount of sodium chloride, which in concentrations above 1 per cent (compare Schwyzer¹⁷) prevented the agglutination

TABLE 11—WASHED CORPUSCLES FROM A NORMAL PERSON (FIBRIN PERCENTAGE IN CITRATED PLASMA 0.21), CITRATED SERUM FROM THE SAME PERSON AND FROM A PATIENT WITH PLEURISY (FIBRIN PERCENTAGE IN CITRATED PLASMA 0.51)

Time After Shaking	2 Cm ³ Normal Corpuscles + 3 Cm ³ Normal Citrated Serum	2 Cm ³ Normal Corpuscles + 3 Cm ³ Pleurisy Citrated Serum
	Sedimentation	Sedimentation
10 min	Traces	Traces
20 min	Traces	Traces
30 min	Traces	<0.1
40 min	Traces	<0.1
50 min	<0.1	<0.1
60 min	<0.1	0.1

When the salt was eliminated by dialysis, the results as shown in Tables 12 and 13 were quite clear and showed that even a low fibrinogen content will increase the sedimentation in physiologic sodium chloride solution

Microscopy of citrated blood has shown that the corpuscles on standing are agglutinated and then settle more quickly. This phenomenon is more pronounced the more fibrinogen the plasma contains.

About these "auto-agglutinins," which cause the agglutination of the corpuscles, we know the following:

(1) Their action varies proportionally with the amount of fibrinogen in the plasma

(2) Their action is extremely weakened, when the fibrinogen is eliminated by spontaneous coagulation

(3) Their action is also weakened when the fibrinogen is eliminated by heat coagulation at 60 C

(4) The pure fibrinogen prepared by repeated precipitation with sodium chloride shows—when freed of sodium chloride—the agglutinating action of these bodies

(5) The serum shows a slight agglutinating action which is stronger in serum whose plasma shows hyperinosis

Since we know these "agglutinins" only by their action, and this is intimately connected with the fibrinogen, one may fancy that it is the fibrinogen and in a lesser degree other plasma proteids which possess these properties

TABLE 12—WASHED CORPUSCLES AND PURE FIBRINOGEN FROM A NORMAL INDIVIDUAL THE FIBRINOGEN IS DISSOLVED IN 0.9 PER CENT SODIUM CHLORIDE (0.19 PER CENT)

Time After Shaking	1.5 Cm ³ Corpuscles + 3.5 Cm ³ 0.9% NaCl	1.5 Cm ³ Corpuscles + 3.5 Cm ³ Fibrinogen Solution
	Sedimentation	Sedimentation
5 min	0	Traces
10 min	Traces	<0.1
15 min	Traces	<0.1
20 min	Traces	<0.1
25 min	<0.1	0.1
30 min	<0.1	0.1

Whether the action of these bodies in the last instance depends on their influence on the electrical charge of the corpuscles is not shown by my experiments

The results of the examination for crusta formation has shown that only in one out of twenty-five women and in no normal man were there found traces of a "buffy coat" on the blood

The woman mentioned had a rather high normal fibrin percentage and a cell volume at the very lowest limit of the normal

TABLE 13—WASHED CORPUSCLES AND FIBRINOGEN FROM A NORMAL INDIVIDUAL THE FIBRINOGEN IS DISSOLVED IN 0.9 PER CENT SODIUM CHLORIDE (0.22 PER CENT) THE EXPERIMENT HAS BEEN MADE ON DOUBLE SPECIMENS

Time After Shaking	1.5 Cm ³ Corpuscles + 3.5 Cm ³ 0.9% NaCl	1.5 Cm ³ Corpuscles + 3.5 Cm ³ 0.9% NaCl	1.5 Cm ³ Corpuscles + 3.5 Cm ³ Fibrino gen Solution	1.5 Cm ³ Corpuscles + 3.5 Cm ³ Fibrino gen Solution
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
5 min	0	0	Traces	Traces
10 min	Traces	Traces	Traces	Traces
15 min	Traces	Traces	<0.1	<0.1
20 min	Traces	Traces	<0.1	<0.1
25 min	Traces	Traces	<0.1	<0.1
30 min	Traces	Traces	0.1	0.1
60 min	<0.1	<0.1	0.2	0.2
Later	0.1	0.1	0.5	0.5

The clinical investigations seemed to show that a crusta phlogistica is formed under two circumstances (1) when the sedimentation of the corpuscles is more rapid than usual, and (2) when the coagulation time is longer than usual

As mentioned above the velocity of sedimentation is increased when the cell volume is low (anemia) or the fibrin (fibrinogen) content of the plasma is increased (hyperinosis)

The velocity of sedimentation also is increased when the temperature of the blood is higher

A determination of the coagulation time of recalcinated plasma (Gram ²⁴) has shown that the changes in this value correspond well with the coagulation time of "natural" blood. A possible exception may be found in some cases of icterus

A lengthening of the clotting time may be found under two circumstances (a) as a result of an alteration in the composition of the blood, and (b) as a result of the conditions under which the blood is taken and kept

In hemophilia and in diseases which are accompanied by thrombopenia (pernicious anemia, lymphatic leukemia, purpura idiopathica and influenzal pneumonia (Gram ²⁴)) the coagulation time may be longer than usual (i e > 6 minutes)

If the blood is kept in a paraffinated glass or at low temperature the clotting time is prolonged

TABLE 14—THE PROPORTIONAL DIVISION OF THE CASES ACCORDING TO THE SIZE OF THE CRUSTA WITHIN THE DIFFERENT DEGREES OF SEDIMENTATION FIVE HUNDRED AND TWENTY-SIX CASES

Sedimentation in 10 Minutes Cm	Crust: Phlogistien			
	— Per Cent	Traces Per Cent	+ Per Cent	++ Per Cent
0	100	0	0	0
Traces	89	8	3	0
0.1	25	37	38	0
0.2	3	20	77	0
0.3	0	6	91	3
0.4	0	13	83	4
0.5	0	6	76	18
0.6 and more	0	7	40	53

A consideration of my material shows with great certainty, that the first factor (sedimentation) is the most frequent and important cause for the formation of a buffy coat

If we arrange the correlated values for sedimentation and crusta formation in classes, we get the results shown in Table 14

Table 14 shows that in some cases no crusta is met with, though the sedimentation is clearly increased. This, however, happens only when the increase is relatively slight (from 0.1 to 0.2 cm in ten minutes)

This may be due to circumstances connected with the taking and the conservation of the blood. A bad venepuncture or unclean glasses may shorten the coagulation time of the blood, and thus prevent the crusta formation

Variations in the room temperature will be compensated partly, since an increase of the temperature makes the sedimentation more rapid, but on the other hand shortens the clotting time

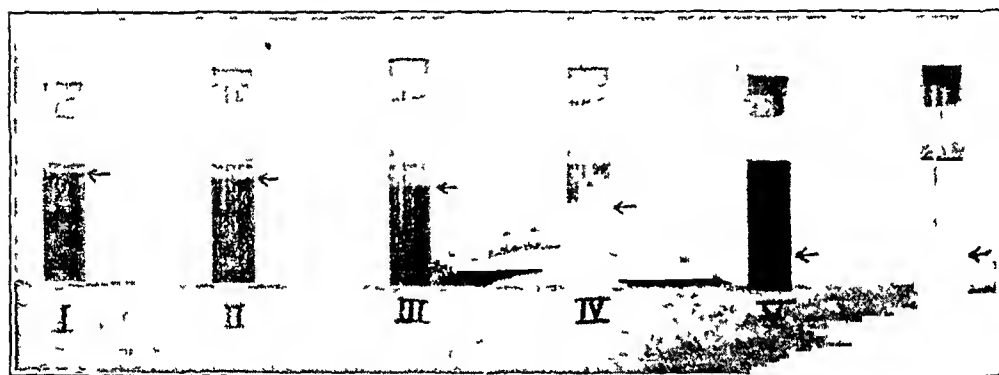
According to Table 14 one finds some cases with only traces (i e, < 0.1 cm) of sedimentation in ten minutes, but with a more or

less pronounced crusta These variations may partly be explained by circumstances connected with the taking and conservation of the blood ²⁹

If we consider the diagnosis in the last named cases we find, however, that nearly all of them may be placed in one of two groups (1) Patients with a high normal or slightly increased fibrin percentage in plasma or a low normal or slightly decreased cell volume, (2) patients with thrombopenia (purpura, pernicious anemia, influenza) or hemophilia

A crusta phlogistica in venous blood, when 1 cm³ is kept at room temperature in a test tube with a diameter of 9 or 10 mm is nearly always a pathologic phenomenon, which may be due to anemia, hypermiosis, thrombopenia or hemophilia

TABLE 15—SEDIMENTATION AFTER ONE HOUR IN SIX NORMAL AND PATHOLOGIC SPECIMENS OF CITRATED BLOOD



Number (from the left)	I	II	III	IV	V*	VI
Diagnosis	Normal man	Normal man	Normal woman	Nephritis	Pernicious anemia	Pneumonia
Sedimentation after 1 hour, Cm	<0.1	0.2	0.4	1.1	2.6	2.8
Cell volume per Cent	49	51	42	47	10	42
Fibrin per Cent in plasma	0.29	0.34	0.27	0.44	0.27	1.31

* Owing to the intense yellow color of the plasma the photograph does not show any difference between plasma and corpuscles in this case (nr V). The border line however was clearly visible to the naked eye and is marked by an arrow on the figure

SUMMARY

1 In a large number (many hundreds) of cases the fibrin percentage in plasma (and blood), the cell volume percentage and sometimes the coagulation time has been determined

29 A small series of experiments has proved that the use of a paraffinated glass may cause crusta formation in the blood of some normal women and even a few men. The best results are obtained when the needle also is paraffinated

2 The fibrin content of the plasma is found to be increased in nearly all infectious diseases, in cancer, nephritis, pregnancy, polyarthritis and after infections of sterile milk. These alterations may all be considered as the effect of the introduction into the circulation of proteids foreign to the blood. A low fibrin percentage in plasma is only found in some, but not nearly all, severe degenerations of the liver.

3 The cell volume, besides in the diseases of the blood proper, is frequently found low, in certain infections, in cancer, pregnancy and polyarthritis.

4 In specimens of citrated blood, the sedimentation has been studied by an observation of the plasma layer formed after ten minutes. Under the conditions described, the formation of a plasma layer of 0.1 cm. or more must be considered distinctly pathologic and is found only in cases showing anemia or hyperinosis.

A clearer analysis of the influence of the fibrin and cell volume percentages may be made by observing the plasma layer formed after one hour. The difference in sedimentation as between normal men and women is shown to depend mainly on the different cell-volumes, less on differences in the fibrin percentage.

5 The formation of the *crusta phlogistica* (in pathologic cases) has been observed in 1 cm.³ of venous blood kept at room temperature in a miniature testtube. The formation of a buffy coat generally coincides with a sedimentation of 0.1 cm. or more in the citrated blood, divergences being found only in border cases and in cases with a lengthened clotting of the blood.

6 From clinical and experimental observations we reach the following conclusions. The formation of a *crusta phlogistica* is a pathologic phenomenon which depends on (a) an accelerated sedimentation, or (b) a lengthened clotting time.

The sedimentation of the corpuscles depends on (1) the fibrin (fibrinogen) percentage in the plasma, the sedimentation being accelerated by a rise in this value, and vice versa. The fibrinogen brings this about by causing an agglutination of the corpuscles, which facilitates their sedimentation, (2) the cell volume percentage, the sedimentation being accelerated by a drop in this value, and vice versa; (3) the temperature, the sedimentation being accelerated when the temperature is higher, and vice versa.

The first two factors may counteract or assist one another, so that a knowledge of one of these, together with a determination of the velocity of sedimentation, allows an estimate of the other factor, that is, if the temperature of the surroundings is constant.

The coagulation time of the blood is found lengthened in hemophilia, and diseases which cause a thrombopenia, i. e., pernicious anemia, lymphatic leukemia, influenzal pneumonia, etc.

This last factor is not nearly so important, practically, as the velocity of sedimentation

However, an accidental shortening of the clotting time may occasionally prevent the formation of a crusta, though the sedimentation is more rapid than normally

The three factors mainly affecting the coagulation time are (1) platelet count, (2) properties of the plasma (hemophilia), and (3) temperature

7 A single specimen of 4.5 cm³ blood taken into an Oluf Thomsen glass (graduated centrifuge tube) allows the determination of the following values (1) platelet count (Oluf Thomsen²²), (2) coagulation time (Gram²⁴), (3) plasma color (Meulengracht³⁰), (4) fibrin percentage in plasma and blood, (5) cell volume percentage, (6) velocity of sedimentation

Another specimen of 1 cm³ allows an observation of the crusta formation

30 Meulengracht *Ugeskr f Læger* **46** 1785, 1919

OBSERVATIONS ON THE RELATION OF UREA TO UREMIA *

LOUIS LEITER
CHICAGO

The objects of this research were to determine what relationship existed between the toxicity of various normal urinary constituents and the manifestations of what is clinically known as uremia—meaning by the term “uremia” the symptoms observed as a result of marked obstruction to the outflow of urine, on the one hand, and, on the other hand, the symptoms which appear in many cases of chronic Bright’s disease, characterized by convulsions, various paralyses and coma

In pursuance of these objects, it was thought advisable to begin with the simplest organic urinary constituent—urea—to determine its toxicity on dogs—when injected in large doses in relatively short periods of time—to note the symptoms produced, to follow the changes in the blood and urine content of urea, and to determine, at the necropsy, the pathologic changes that might have taken place—both gross and microscopic—as well as the urea content of various body fluids. Then, the toxicity of urea was determined in dogs in which one ureter had been ligated, and in other cases both ureters were ligated and the changes in the blood urea followed until the death of the animal

THE LITERATURE

There is a tremendous literature¹ on the subject of uremia and related topics, but, while theories are abundant, and there are many facts, there is a great deal of conflict of opinion on every aspect of the subject—even on the subject as to what constitutes “uremia”

One of the earliest theories of the causation of uremia was a chemical theory put forth by Wilson in 1833, in which the symptoms were ascribed to the retention of urea in the blood. But discrepancies were soon noted between the amounts of urea in the blood and the severity of the symptoms in patients, and later workers, Frerichs, in 1852, Feltz and Ritter, in 1880, Bouchard, in 1887, Herter, in 1898, and many others, found that urea was relatively nontoxic, when injected into animals. In fact, Bouchard, using rabbits, found that the amount of a concentrated urea solution necessary to produce severe symptoms was equal to the toxic dose of distilled water itself. Although Gallois, Richardson, in 1862, Voit, in 1868, and others, claimed positive results,

* From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute

1 For most of the literature up to 1900 we have made use of Ascoli’s “Uramie” (1902) in which a complete review of the subject is given

they were met with the objection that their results were due, not to the urea itself, but to various impurities or to the osmotic changes induced by the concentrated solutions employed

An anatomic theory, suggested by Traube in 1860, ascribed uremia to edema of the brain and consequent circulatory disturbances. But necropsies soon disclosed the fact that great inconsistencies existed between the anatomic and clinical findings, so the theory lost ground, and a return to a chemical theory followed. This time ammonium carbonate was blamed, Frerichs, in 1851, postulating that urea in the blood was converted by ferments into the toxic ammonium compound, while Treitz, in 1859, believed that the change occurred in the intestines. However, the ammonia content of uremic blood was found to be negligible.

Voit, in 1868, Feltz and Ritter, in 1881, and Linbeck, in 1898, turned their attention to the inorganic constituents of urine, and chose potassium chlorid, because of its extreme toxicity to animals, as the cause of the symptoms of uremia—especially the convulsions and coma. However, the clear-cut cardiac picture in potassium poisoning, and the inability to demonstrate a constant increase in the potassium content of the uremic blood, spoke against this theory. Schottin, in 1853, found an increased amount of extractives in the blood of uremics—especially creatin and creatinin. Jaccoud, in 1867, had a similar experience, and Landois, in 1891, had worked up the subject very thoroughly and produced convulsions in rats, dogs and apes by the direct application of creatin to the cerebral cortex. Feltz and Ritter, however, had to inject 2 gm creatinin intravenously before they could produce convulsions and death, and these they obtained only in one dog. Various salts of creatin and creatinin, in from 5 to 6 gm doses, had no effects. Various other extractives—uric acid, hippuric acid, leucin, tyrosin, purin bases, phosphates, sulphates and chlorids—were similarly ruled out as a possible cause of uremia.

Still clinging to the idea of urinary retention as the cause of uremia, Bouchard, from 1882 to 1889, worked on the toxicity of whole urine when injected into rabbits. He found human urine quite toxic, and could account for only half of this toxicity on the basis of potassium salts. He could extract various substances from human urine, which, on injection into rabbits, produced any or all of the symptoms of uremia. The urine of nephritic patients was found to be much less toxic than normal urine—due, presumably, to retention of the toxic products. Bouchard calculated that in twenty-four hours an average man excreted sufficient toxins in his urine to kill him in fifty-two hours, were all these toxins not eliminated. But Fowler, in 1881, reported many cases of complete mechanical obstruction to the out-flow of urine, the patients living for many days and even two weeks without any other symptoms than asthenia and drowsiness. Hence,

there was a great difference between uremia and anuria. Bouchard's work, on the whole, was very crude, and it was to be expected that he could obtain almost any symptoms by injecting such a complex of unknowns and unknowables as is present in human urine.

Brown-Sequard, in 1889, published his theory on internal secretions and included the kidney as one of the organs of internal secretion, ascribing uremia to failure or lack of such a secretion. In 1893, he stated that while nephrectomized animals lived only thirty hours on the average, similar animals to whom kidney extracts were given lived sixty hours. Meyer, in 1893 and 1894, reported that renal extracts had a marked effect on the pulse and respiration of anesthetized animals that were nephrectomized, changing the respiration from Cheyne-Stokes to normal. These results were obviously trivial and unimportant, and the theory of internal secretion of the kidney was dropped for a time.

Ascoli could see no very close relationship between the nonprotein nitrogen of the blood and the severity of symptoms, although some parallelism might be made out. Perhaps, both are merely the results of nephritis and, hence, a causal relationship between the two need not exist. In 1902, Ascoli described nephrolysins—substances having specific toxic effects on the kidneys, with side effects on nervous tissue, substances akin to immune bodies and produced by using renal tissue as antigen—and even isonephrolysins, produced when a kidney is injured for some reason or other,—and explained all the symptoms of what he called “true uremia without retention”—in contrast to “urinary intoxication with retention”—on the basis of a general intoxication with nephrolysins. He believed that nephrolysins could cause the increased blood pressure, the localized palsies, the edema, the pericarditis, stomatitis and colitis, the convulsions, the coma—in short, the classical picture of a clinical uremic attack. Ascoli emphasized especially the difference between the uremia resulting from mechanical obstruction of the urinary tract and the uremia found as a terminal event in cases of chronic nephritis—something which previous investigators had not quite clearly understood, and which was the cause of much unnecessary dispute and inconsistent experimental work. Every author had a different idea of uremia, and it is no wonder that no two authors agreed in their results.

With the advent of physicochemical investigations, many men turned their attention to the relationship between physicochemical changes in the blood and uremia. Honigmann² reviewed the literature in 1902. Koranyi found that the molecular concentration of the blood increased in uremia—using cryoscopy. Lindemann found a definite increase in the depression of the freezing point of water (Δ) of the blood of

² Honigmann *Ergeb d Pathol* 8 549, 1902

uremic patients and dogs with both ureters ligated, and believed that the change in osmotic pressure was the chief cause of trouble. In an exhaustive monograph, Strauss, in 1902, found an increased Δ of the blood—usually in chronic interstitial nephritis—and a retention of organic molecules till the limit of the patient's tolerance to poisonous metabolites was reached, when any one or several of many factors might produce the uremic intoxication. Bickel, in 1902, found no change in the electrical conductivity of the blood of nephrectomized animals, and, with an increased Δ , he concluded that there must be a retention of organic molecules. However, in all these cases, no constant relationship could be found between the changes in Δ and other physical constants and the severity of the symptoms, so that, as Honigmann correctly concluded, no single series of facts—chemical, physical, or otherwise—had yet stood in constant parallelism with the clinical picture of uremia. The new findings were simply concomitant symptoms, so to speak, and not etiologic factors. Yet Stern³ was willing to ascribe uremia to diminished electrical conductivity of the blood serum.

Bradford, in 1899, excised varying amounts of renal tissue in dogs, and found an increased output of nitrogen in the urine—due, he thought, to increased nitrogenous catabolism in the muscles. Others misinterpreted his views as favoring an internal secretion for the kidney. Pearce,⁴ in 1908, removed one half, two thirds and three fourths of the renal tissue in dogs, but found no change in nitrogenous metabolism. But removal of more than three fourths of the total renal tissue led to metabolic upset, owing to starvation and gastro-intestinal disturbances. This work was well controlled, and the further work of Pearce⁵ and Pearce and Sawyer⁶ helped to disprove completely two theories—the internal secretion theory and Ascoli's nephrolysin hypothesis. Pilcher, in 1913,⁷ extirpated renal tissue by ligation of the renal vessels—instead of excision of kidney tissue—and obtained results entirely confirmatory of Pearce's work.

When theories begin to fail, people turn their attention to facts. In scientific work much more progress may be made by the invention of new methods than by modifications of old ones, and so, in the work on uremia and chronic nephritis, the development of, perhaps, the most interesting phase of the problem—the retention or accumulation of waste products, especially nitrogenous, in the blood—was made possible by the new, delicate methods of blood and urine analysis.

3 Stern *Med Rec* **63** 121, 1903

4 Pearce *J Exper M* **10** 632, 1908

5 Pearce *Arch Int Med* **2** 77 (July) 1908

6 Pearce and Sawyer *J M Res* **19** 269, 1908

7 Pilcher *J Biol Chem* **14** 389, 1913

introduced by Folin and his collaborators⁸ since 1912, and extended by Marshall,⁹ Van Slyke and Cullen,¹⁰ Myers and Fine,¹¹ and many others. As a result, much of the older work has been discounted, and advantage is taken of the new methods by clinicians and laboratory men alike, in order to help accumulate sufficient data from which to draw definite conclusions.

It was suspected very early in the nineteenth century that a retention of nitrogenous waste products occurred in the blood in nephritis. The methods of investigation, however, were very crude, and the results inaccurate. Among those who worked on the nitrogen content of the blood of uremic patients, ordinary nephritics and experimental animals, were Wilson, Bostock, Frerichs, Treitz, Ascoli, Van Noorden, Schotten, Oppler, Hoppe-Seyler, Jaccoud, Landois, and a host of others.¹² Strauss, in his monograph, gave the nonprotein nitrogen of normal blood as from 20 to 35 mg per 100 c c, in chronic parenchymatous nephritis, 40 mg or over, in chronic interstitial nephritis, 85 mg and over, reaching higher values, in uremia. Hohlweg, in 1911,¹³ found 41 to 60 mg of "residue" nitrogen per 100 c c in normal sera, of which 60 per cent was urea nitrogen, as high as 120 mg in nephritis, and up to 340 mg in uremia. Other conditions showed no such changes. Foster, in 1912,¹⁴ found from 43 to 164 mg nonprotein nitrogen in chronic nephritis, from 53 to 393 mg in uremia, with the urea nitrogen varying from 36 to 90 per cent of the nonprotein nitrogen. Nonprotein nitrogen of 100 mg (per 100 c c) or more was considered a poor prognostic omen.

With newer methods, the following results were obtained. Folin and Denis, in 1913,¹⁵ found, in normal people, nonprotein nitrogen from 22 to 26 mg, urea nitrogen, from 11 to 13 mg, in chronic nephritis, nonprotein nitrogen of from 40 to 96 mg, urea nitrogen of from 19 to 68 mg. Farr and Austin, in 1913,¹⁶ found, in nonnephritic cases nonprotein nitrogen from 15 to 43 mg with urea nitrogen of from 50 to 60 per cent of the former, no change in uncomplicated cardiovascular cases, some increase in chronic parenchymatous

8 Folin and Farmer *J Biol Chem* **11** 493, 1912. Folin *Ibid* **11** 507, 1912. Folin and Macallum *Ibid* **11** 523, 1912. Folin and Denis *Ibid* **11** 527, 1912.

9 Marshall *J Biol Chem* **14** 283, 1913, *ibid* **15** 487, 1913, *ibid* **15** 495, 1913.

10 Van Slyke and Cullen *J Biol Chem* **19** 211, 1914, *ibid* **24** 117, 1916.

11 Myers and Fine *J Biol Chem* **20** 391, 1915.

12 Obermayer and Popper *Ztschr f klin Med* **72** 332, 1911.

13 Hohlweg *Deutsch Arch f klin Med* **104** 216, 1911.

14 Foster *Arch Int Med* **10** 414 (Sept) 1912.

15 Folin and Denis *J Biol Chem* **14** 29, 1913.

16 Farr and Austin *J Exper M* **18** 228, 1913.

nephritis, nonprotein nitrogen from 40 to 180 mg in chronic nephritis with hypertension. The nonprotein nitrogen was usually, but not constantly, high in uremia. Tileston and Comfort, in 1914,¹⁷ studied 142 cases of nephritis and many other conditions—the latter as controls—and considered as the normal nonprotein nitrogen 30 mg or less, with 50 per cent urea nitrogen. In uremia, the nonprotein nitrogen was as high as 324 mg, urea nitrogen, 70 per cent or more. Nonprotein nitrogen over 100 mg was practically never found by them in conditions other than uremia, when occurring it was a very serious sign, and was apt to be followed very soon by death. Patients with a high nonprotein nitrogen were poor surgical risks.

McLean and Selling, in 1914,¹⁸ gave the normal nonprotein nitrogen as from 23 to 36 mg per 100 c c, the urea nitrogen as from 10 to 23 mg. Foster, 1915,¹⁹ found an average nonprotein nitrogen of 63 mg in parenchymatous nephritis, with 84 mg in the chronic interstitial variety, and 135 mg in "convulsive uremia." The uric acid—normally from 3 to 5 mg per 100 c c—averaged 12 mg in uremic patients, but there was no concordance between the rise in purine and nonprotein nitrogen. In four convulsive cases, the creatin and creatinin ran from 27 to 46 mg per 100 c c, indicating a rather marked retention. Cullen and Ellis, 1915,²⁰ compared the urea nitrogen in blood serum and spinal fluid respectively, and found in the former a range of from 20 to 42 mg, in the latter from 22 to 46 mg, a very close correspondence, it is seen, and indicating the marked diffusibility of urea.

Schwartz and McGill, 1916,²¹ studying the blood urea, gave, as the normal for human blood, 25 mg urea per 100 c c, in acute nephritis, twenty-five cases, an average of 57.8 mg was obtained, in mild cases of chronic nephritis, 28.8 mg with 44.4 mg in advanced cases, and 52.3 mg in advanced cases with complicating cardiac decompensation. The urea content of blood and other body fluids was found to be very similar. Kast and Wardell,²² 1918, in a study of 244 hospital cases with a urea nitrogen of less than 35 mg, concluded that 20 mg was the upper normal limit for blood urea nitrogen, thus agreeing pretty closely with most of the previous figures. Finally, Gettler and St. George,²³ 1918, in a review of 600 cases of primary nephritis, found the nonprotein nitrogen to range between 40 and 460 mg as compared with the normal of from 25 to 40 mg, while the

17 Tileston and Comfort. *Arch Int Med* **14** 620 (Nov.) 1914.

18 McLean and Selling. *J Biol Chem* **19** 31, 1914.

19 Foster. *Arch Int Med* **15** 356 (Sept.) 1915.

20 Cullen and Ellis. *J Biol Chem* **20** 511, 1915.

21 Schwartz and McGill. *Arch Int Med* **17** 42 (Jan.) 1916.

22 Kast and Wardell. *Arch Int Med* **22** 581 (Oct.) 1918.

23 Gettler and St. George. *J A M A* **71** 2033 (Dec. 21) 1918.

urea nitrogen, normally from 10 to 18 mg, ranged from 20 to 375 mg. In 120 cases of gout, the nonprotein nitrogen was from 38 to 55 mg and urea nitrogen from 15 to 35 mg—somewhat increased. They stated that where the nonprotein nitrogen was 80 mg or above, increased protein catabolism, or failing, or poor, circulation could not be blamed as the cause, but the only possible cause was some renal trouble.

An interesting fact was pointed out by Chace and Myers,²⁴ 1916, who showed that in the various stages of a clinical nephritis, uric acid was retained first, then urea and, last of all, creatinin, so that one might have uric acid from 61 to 95 mg with normal urea and creatinin, giving a "steplike" picture. In fatal cases, the uric acid ranged from 87 to 224 mg, urea nitrogen from 144 to 263 mg, and creatinin from 110 to 222 mg. All patients with a blood creatinin of more than 5 mg died in a short time. They record thirty-four cases in which the creatinin ranged from 49 to 333 mg. Myers and Killian,²⁵ 1916, reported ninety-four cases with creatinin values of more than 5 mg, of which number eighty-three patients had died in a short time—80 per cent in less than two months. They believed that the blood creatinin determination furnished a most reliable prognostic test in nephritis or uremia.

From the experimental side, it is interesting to note that in uranium nephritis—in cats—a marked retention of nonprotein nitrogen and urea nitrogen occurs, so also in cantharidin nephritis and less so in chromate nephritis, as reported by Folin, Karsner and Denis,²⁶ in 1912. Later on, in 1914, Karsner and Denis²⁷ produced a marked retention in acute nephritis as the result of injections of specific hemolytic immune serum, diphtheria toxin, and, especially, tartaric acid—nonprotein nitrogen 382 mg in one cat. Tubular changes seemed a little more important in producing retention than glomerular changes, but definite conclusions could not be drawn. Removal of from one half to two thirds of the total kidney substance resulted in only a slight nitrogen retention for from twenty-four to seventy-two hours, but complete nephrectomy was followed by a progressive increase in nonprotein nitrogen of the blood—the values ranging from 227 to 285 mg in three dogs reported by Karsner and others,²⁸ 1915.

Another evidence of retention in chronic nephritis and uremia is afforded by the study of the blood indican. Obermayer and Popper,¹² in 1911, believed that the presence of indican in the blood was specific

24 Chace and Myers *J A M A* **67** 929 (Sept 23) 1916

25 Myers and Killian *Proc Soc Exper Biol & Med* **16** 41, 1918

26 Folin, Karsner and Davis *J Exper M* **16** 789, 1912

27 Karsner and Denis *J Exper M* **19** 259, 1914

28 Karsner, Bunker and Grabfield *J Exper M* **22** 544, 1915

for uremia In thirty-eight uremic patients they found a blood indican of from 37 to 60 mg per 100 c c They did not ascribe any etiologic rôle to indicanemia, but considered it a delicate indicator of retention Tschertkoff,²⁹ in 1914, found indicanemia in many cases of nephritis without uremia Haas,²⁹ in 1914, using better methods, determined 0.045 mg per 100 c c as the amount of indican in the blood of normal individuals, from 0.06 to 0.145 mg occurring in gastro-intestinal affections, from 0.2 to 2.7 mg in nephritis with definite "renal insufficiency" Indican was increased long before the nonprotein nitrogen and was a better indicator of impending "renal insufficiency" than either the nonprotein nitrogen or urea nitrogen—a value of 0.180 mg being a very suggestive sign of impending trouble However, injection of large doses of indican into dogs and man—up to 40 mg—proved perfectly harmless, as might be expected

It was early discovered that in the late stages of renal diseases there occurs a state of acidosis, or decreased alkali reserve of the blood Jaksch,² in 1900, found a decreased alkalinity of the blood in uremia Orlowsky,² 1902, found a similar change in dogs with both ureters ligated This was considered as simply another evidence of retention Straub and Schlayer³⁰ believed acidosis to be an important factor in the production of uremia, for they obtained an alveolar carbon dioxide tension of less than 35 mm Hg, in eight cases of uremia No acetone bodies were found Peabody,³¹ in 1915, found an acidosis—determined by the bicarbonate tolerance method of Sellards—in advanced cases of chronic nephritis, in very advanced cases the alveolar carbon dioxide partial tension fell This acidosis was secondary to retention, and very constant in uremia In only a few cases, however, did it cause a definite air-hunger or other symptoms Sellards³² had previously obtained very similar findings Somewhat at variance with other investigators, Fischer,³³ in 1912, went as far as to call acidosis the cause of nephritis In 1916, Marriott and Howland,³⁴ following the finding by Greenwald of increased total phosphorus in the serum of nephritics, reported fourteen cases of chronic nephritis and thirty-five normal people on whom blood studies had been made regarding the nonprotein nitrogen, urea nitrogen, inorganic phosphorus, calcium and magnesium In normal people, the total inorganic phosphorus was usually less than 2 mg per 100 c c, in

29 Haas *Deutsch Arch f klin Med* **119** 117, 1916

30 Straub and Schlayer *Munchen med Wchnschr* **59** 569, 1912

31 Peabody *Arch Int Med* **16** 955 (Dec) 1915

32 Sellards *Bull Johns Hopkins Hosp* **23** 289, 1912, *ibid* **25** 141, 1914, *Principles of Acidosis*, 1917

33 Fischer *Edema and Nephritis*, 1912

34 Marriott and Howland *Arch Int Med* **18** 708 (Dec) 1916

chronic nephritis without acidosis, it was from 27 to 55 mg, in chronic nephritis with acidosis, it ranged from 8 to 23 mg. They concluded that acidosis in nephritis was due to retention of acid phosphates—a condition not found in diabetic acidosis. The rather low calcium content found might be related to the convulsions and hemorrhages of uremia, the increased phosphorus probably caused an increased excretion of calcium phosphate into the intestine.

As regards other changes in the blood, than those described above, Gabbi² described an increased serum globulin in rabbits and dogs with both ureters ligated. Recently, in 1916, Butterfield and others³⁵ studied the blood of normal people and those with cardiac and renal decompensation and edema, uremia and hypertension, for the Δ , refractive index, specific gravity, dry residue and protein content. In chronic nephritis with uremia, Δ was from 0.61 to 0.76 degrees—quite high as compared with the normal Δ —0.57, the dry residue was increased, and especially the total protein content, i. e., the serum was concentrated. When edema was also present, all the findings but Δ were lowered, due to dilution. Cases of cardiac decompensation with edema showed normal values. Rowe,³⁶ in 1917 found, in chronic nephritis with edema, the lowest values for total serum proteins of those found in any disease, however, the globulin was increased. Bienenstock and Csaki,³⁷ in 1917, made an extensive series of observations on the physicochemical changes in the blood of dogs in whom both ureters were ligated or both kidneys removed. Δ was increased in all cases, up to 0.771 degrees, the electrical conductivity of the serum was decreased, the organic molecules were increased, the serum ash was increased, the fluidity of the blood varied, as also the viscosity, surface tension and total solids. The carbon dioxide in the serum was decreased, but markedly increased in the red blood corpuscles. The H-ion concentration of the serum rose—due to undetermined organic acids.

Two suggestive studies have been made on the presence of some very toxic substance in the blood or urine. Foster,³⁸ in 1915, isolated a toxic base from the blood of seven uremic patients—not found in twelve controls—which caused death in guinea-pigs, preceded by dyspnea, twitchings, convulsions, coma and subnormal temperature. At the necropsy, there were found hyperemia of the brain and kidneys. Hartman,³⁹ in 1915, isolated an organic compound, C_6H_5O , which he called "urinod," obtainable from urine, from 1 to 2 parts in 100,000.

35 Butterfield et al. *Am J M Sc* **151** 63, 1916

36 Rowe. *Arch Int Med* **19** 354 (Feb) 1917

37 Bienenstock and Csaki. *Biochem Ztschr* **84** 210, 1917

38 Foster. *Tr Assn Am Phys* **30** 305, 1915

39 Hartman. *Arch Int Med* **16** 98 (July) 1915

Urinod was very toxic, producing symptoms very much like those of uremia, and having the typical "urinous" odor of a uremic patient's breath. The symptoms reproduced in man and animals were chiefly of the nervous type.

The clinical classification of uremia has varied from time to time as new facts were established and old ones confirmed or disproved. Thus, Widal,⁴⁰ in 1912, described two chief types of uremic syndromes—the "chloruremic" variety, associated with retention of sodium chlorid and with symptoms of edema, diarrhea, vomiting, coma, Cheyne-Stokes breathing, delirium, convulsions and amblyopia, and the "azotemic" type in which nonprotein nitrogen retention was marked, with gastrointestinal disturbances—*anorexia*, vomiting, ulcerative stomatitis and colitis, pericarditis, albuminuric retinitis, stupor and torpor—with mixed pictures resulting from the predominance of urinary, vascular or cardiac syndromes.

Reiss,⁴¹ in 1914, in an excellent review of the subject, attempted to classify uremia on a symptomatic basis, and gave three main types—the "asthenic uremia," in which predominating symptoms were drowsiness, fatigue, apathy, indifference, physical weakness, marked nonprotein nitrogen retention, and death, usually from rather sudden cardiac failure, the "convulsive or epileptiform" type, in which convulsions, unconsciousness, a high blood pressure, with little or no disturbances in the urinary secretion and practically no nitrogenous retention were characteristic symptoms, the "psychotic uremia," in which psychic symptoms, such as dementia, hallucinations, delusional insanity, deep coma and muscle twitchings, occurred, with nonprotein nitrogen not necessarily increased, but with evidence of cerebral arteriosclerosis in the majority of cases, and, finally, a mixed group in which asthenic and convulsive features were combined—by far the largest clinical group. In the asthenic variety alone were nonprotein nitrogen and sodium chlorid retention marked and characteristic. Reiss believed that the nonprotein nitrogen retention was the cause of asthenic uremia. In the other types, it could be stated definitely that the toxic substances did not arise from disturbances in the urinary tract, but were metabolic in origin.

Strauss,⁴² in 1915, reserved the term "true uremia" for cases with definite retention—nonprotein nitrogen, 150 mg or more. All the other forms, including the convulsive and arteriosclerotic types, he grouped under "pseudo-uremia"—with nonprotein nitrogen below 70 mg. Most authors now agree in recognizing at least two types of uremia—the

40 Widal. *Presse med* 20 973, 1912.

41 Reiss. *Ztschr f klin Med* 80 97, 1914.

42 Strauss. *Berl klin Wchnschr* 52 368, 1915.

asthenic and the toxic Heim and Tchertkoff,⁴³ in 1918, described three types—one due to chlorid retention, a “true uremia” due to urea retention, and “pseudo-uremia”—the latter associated with general arteriosclerosis, and no retention, usually found after 40 years of age.

The finding of increased nonprotein nitrogen in the blood of uremic patients, and the fact that from 30 to 90 per cent of this nitrogen was urea nitrogen, naturally led to the thought that urea was toxic. Experimentally, it was shown that double nephrectomy or ligation of the ureters produced a rise in blood urea. Thus, Herter and Wakeman,⁴⁴ in 1899, removed both kidneys in the dog, with resulting subnormal temperature, anorexia, drowsiness, vomiting and diarrhea, and death in from twenty-two to eighty-two hours. Ligation of both ureters gave similar results. In twenty-eight normal dogs, the blood urea was 37 mg (average), in eighteen nephrectomized animals, it was as high as 315 mg (average), and 307 mg in animals with both ureters ligated. There were also an increase in the total proteins of the blood, and in the total phosphorus. The tissues, in general, contained from four to five times the normal percentage of nitrogen—probably urea. It might be said that earlier work on the subject is open to criticism because of the great inaccuracy of the analytical methods in vogue.

In 1914, some definite work on the toxicity of urea was done by Marshall and Davis,⁴⁵ using accurate but simple methods. Vauquelin and Segales, in 1822, were the first to record intravenous injections of urea in animals. They used small amounts and obtained negative results. Hammond, in 1858, interfered with the kidneys while injecting, and found the injections fatal. Grehant and Quinquard, in 1884, determined 600 mg per 100 c c blood as the lethal dose for dogs. Herter and Wakeman, in 1899, caused the death of normal dogs by using concentrated urea solutions, urea to the extent of 1 per cent of the body weight had to be injected to produce fatal effects. Marshall and Davis studied the distribution of injected urea, in the blood, urine, bile, spinal fluid and tissues. In normal dogs, the blood urea was from 21 to 29 mg per 100 c c, while the urea content of other body fluids and tissues was very similar to these figures, except in the case of fat, which was poor in urea, and the urinary tract, which was obviously rich in urea.

By injecting increasing doses of urea, in concentrated solutions, into dogs on successive days they produced, in the order named, loss of appetite, vomiting and restlessness, convulsions, coma and dyspnea, and death—the latter occurring when the amount injected reached 1 per cent of the body weight, e g, 25 gm in a 27 kg dog. The urea

43 Heim and Tchertkoff. *Rev. med. Suisse Rom.* **38** 15, 1918.

44 Herter and Wakeman. *J. Exper. M.* **4** 117, 1899.

45 Marshall and Davis. *J. Biol. Chem.* **18** 53, 1914.

injected was excreted rapidly, and what remained was fairly evenly distributed in the blood and tissues of the body—as one would expect from the ready diffusibility of urea. There was no evidence that any of the urea was changed into other compounds.

In some animals one ureter was ligated, and it was found that, after a short time, the remaining functioning kidney could take care of as much urea as both kidneys previous to the operation, indicating a high margin of safety.

It was shown, both by injecting single large doses and repeated small doses of urea, that the tissues took up about 90 per cent of the injected urea in a few minutes. Tremors and opisthotonic convulsions were produced when large doses were injected. At the necropsy, the brain was found to be wet and hyperemic.

Marshall and Davis concluded that the concentration of urea in the blood did not affect the ability of the tissues to absorb urea, that the kidneys had an enormous power of excreting urea—up to 40 gm per liter, that dehydration retarded elimination, and that, in man, urea accumulated only slowly in the blood, owing to absorption by the tissues, so that it would take from four to six days of complete retention to raise the blood urea to 200 mg. Analysis of human tissues, from cases of nephritis, showed a high urea content, closely analogous to that found in the dogs. Urea was toxic only in large doses—1 per cent of body weight—but produced symptoms in smaller amounts. It was not transformed into other compounds.

As regards the toxicity of urea in man, when urea is ingested, Hewlett and others,⁴⁶ in 1916, carried out some interesting experiments. One hundred gm of urea, in aqueous solution, were swallowed in fifteen minutes, or in divided doses, over a period of from four to six hours. Symptoms of headache, dizziness, apathy, drowsiness, fatigue and inability to work appeared, and were most marked when the blood urea was at its maximum—from 150 to 160 mg per 100 cc. There were practically no symptoms between 40 and 150 mg, and symptoms always appeared after 150 mg, persisting till the blood urea had again fallen below that level. Hence, the sudden entrance of urea into the blood was not the cause. The authors noted the analogy between the symptoms produced and those seen in asthenic uremia, or in the later stages of nephritis when retention occurred.

Following a thorough review of the literature on uremia, Wells,⁴⁷ in 1920, was inclined to the view that uremia was caused by the retention of the known nitrogenous substances in the blood, emphasizing especially the importance of the time element, for the presence in the blood of a high concentration of organic substances, continuously main-

⁴⁶ Hewlett, Gilbert and Wickett. *Arch Int Med* **18** 637 (Nov.) 1916.

⁴⁷ Wells. *Chemical Pathology*, 1920.

tained, was bound to have deleterious effects, especially when one considered the marked symptoms following even a very temporary increase in nonprotein nitrogen, if 200 mg or more. All the pathologic findings pointed to a systemic intoxication, and varying clinical pictures might very readily be produced by variations in the retention of the several known nitrogenous constituents. Experiments with the Wood-yatt machine to determine the effects of prolonged intravenous injections of the various purified urinary constituents were recommended as being highly essential for further progress.

THE METHODS OF EXPERIMENTATION

Dogs were used. They were not restricted as to food or drink. Injections of urea were made intravenously, using a buret, rubber tube and hypodermic needle, usually without cutting down on the vein—the external saphenous or some superficial thigh vein—unless absolutely necessary, and then under local anesthesia. A 33½ per cent solution of urea was always used, at body temperature. No general anesthetic was necessary since the dogs lay quietly on the table, except when convulsions occurred. Blood was drawn from a leg vein, by means of a hypodermic needle attached by short rubber tubing directly to an Ostwald pipet, so that suction could be applied. In the first few dogs the urine was obtained by means of a catheter, but later on, owing to the difficulty of inserting a catheter without assistance, and because it was not possible always to obtain female dogs, the catheter was abandoned, and samples of urine obtained only during urination and at necropsy. No attempt was made to collect twenty-four hour samples of urine.

The injections were usually continuous, except for the time it took to fill a 50 cc buret, and were continued until the twitchings or convulsive movements of the animal made injection difficult. Symptoms were noted carefully, at varying intervals. Necropsy was performed on all the dogs within a few hours after death, and samples of heart's blood, urine, bile, gastric or intestinal contents, taken for analysis. The viscera and the brain and spinal cord were examined in a routine manner and hematoxylin and eosin celloidin sections, as well as frozen sections for fat stains, were made.

In cases in which operative procedures were necessary—in ligating the ureters—strict aseptic precautions were observed and the anesthetic, ether, was administered by an assistant. A midline abdominal incision was employed, except in one case.

The urea in the blood was determined by mixing the drawn blood with 1 cc 5 per cent sodium citrate and diluting, or not, with distilled water, if necessary, so as to give from 1 to 3 mg of urea in 5 cc of the mixture or whole blood. In a large test tube, 5 cc of whole or diluted blood were placed, 5 cc of water added, 1 cc of 10 per cent urease solution (freshly prepared from "Arlco" urease) and a few cc of paraffin oil (later a few drops of caprylic alcohol were substituted), and allowed to stand at room temperature for at least 30 minutes. This tube was connected with another tube containing from 15 to 25 cc of hundredth normal hydrochloric acid, some 1 per cent solution of sodium alizarin sulphonate, and paraffin oil as above—the set of two tubes constituting part of a complete aeration apparatus, with a water suction pump to create a negative pressure. Usually, several sets of tubes were run in series. After standing thirty minutes, suction for a minute or two was carried out to get any free ammonia vapor into the acid tube, then 10 cc of a 50 per cent suspension of anhydrous, finely-powdered sodium carbonate were introduced into the tube containing the fluid to be analyzed, and suction carried out for at least thirty minutes, at first a slow current for from two to three minutes and then a gradually increasing current until the maximum

aeration was obtained Titration of the acid tube with hundredth normal sodium hydroxid and sodium alizarin sulphonate, as indicator, was done, and the urea readily estimated

For urine, the ammonia content was first determined by simply adding 10 cc of a 15 per cent potassium carbonate and 15 per cent potassium oxalate mixture to 1 cc of urine, and aerating The ammonia nitrogen was subtracted from that found in the estimation of urea Urine was usually diluted 1 100, and so also bile and other body fluids, before determining the urea content

Experiments with known solutions of urea were carried out until accurate results were obtained with the urease and titration method Controls were, of course, run on the blood and other fluids for ammonia nitrogen not derived from urea, and all the reagents were carefully controlled, so that the figures given below represent the completely corrected figures Urea was usually injected at the rate of 2 gm per minute

RESULTS

Owing to lack of space, the protocols of only a few of the experiments in each series are given in detail, while the remainder are abbreviated so as to include only the essential features of each experiment and any points of difference in the results of the various experiments carried out

April 11, 1920—Dog 1, brown female, 95 kg, received 30 gm urea intravenously, vomited a few times Blood urea rose from 207 mg per 100 cc to 348.3 mg No after-effects were noticed

April 11, 1920—Dog 2, white female, 163 kg, showed no symptoms during or after the injection of 20 gm urea, while the blood urea rose from 246 mg to 1614 mg per 100 cc

April 15, 1920—Dog 2 received 80 gm urea Nausea and salivation occurred after 40 gm urea were injected, dyspnea and vomiting after 70 gm Blood urea rose from 227 mg to 4914 mg Urine urea was 1,780 mg per 100 cc at the end of the experiment The animal seemed well the next morning

April 26, 1920—Dog 2, white female, weighing 166 kg, previously injected 4 30 p m—Normal blood urea, 184 mg, temperature 102.2 F

4 50-5 17 p m—Injected 65.3 gm urea (Merck) At 20 gm dog was panting markedly (240 per minute), at 40 gm salivation, nausea and vomiting, with dyspnea Temperature 102.0 F

5 30 p m—Blood urea, 5364 mg

5 47-6 15 p m—Injected 60 gm urea

5 50 p m—Dog quiet, respiration, 14 (per minute), heart, 66

6 00 p m—Dog grunting, respiration 48, heart, 102, temperature, 101.4 F

6 30 p m—Urine bloody, no casts, 5,090 mg urea per 100 cc urine

6 55-7 15 p m—Injected 77 gm urea Total injected 202.3 gm

7 01 p m—Dog dyspneic (144 resp), very restless and irritable

7 15 p m—Blood urea, 1,812 mg

7 25 p m—Generalized tremors

7 30 p m—Temperature, 97.2 F, tonic, then clonic convulsions, affecting the whole body, lasting thirty minutes, then spasmodic jerking of the head and limbs, with paddling movements of the forelimbs for at least three hours Frequent urination and bloody diarrhea, the animal was unconscious, the reflexes exaggerated, the breathing jerky and rapid

9 00 p m—Temperature below 94 F

April 27, 1920—8 00 a m The dog still has jerky, opisthotonic movements of the head and neck, is in deep coma with slow respiration and slow pulse, no corneal reflexes, dilated pupils

10 00 a m—Similar condition, deep coma, slow twitchings Died between 11 and 12 a m

1 00 p m—*Necropsy*—The whole small intestine was deeply injected, the mucosa of the stomach and small intestine was swollen, hemorrhagic and necrotic, the colon hyperemic, the kidneys large, tense, bluish with the cut surface pale and swollen, with fine grayish streaks in the lower half of the cortex, the medulla glistening, the heart with the right ventricle dilated, with subendocardial petechiae, the lungs collapsed, not crepitating slightly boggy, multiple subpleural hemorrhages, the liver dark and full of blood, the gall-bladder distended with thick tarry bile, the brain and cord hyperemic and wet, the urinary bladder empty

Histologic examination showed marked fatty degeneration (Sudan III frozen sections, hematoxylin as counter stain) in the limbs of Henle's loops and in the collecting tubules, with cloudy swelling of the convoluted tubules, central congestion and fatty degeneration in the liver, hyperemia of the intestinal mucosa, with mucoid degeneration of the epithelium and marked edema of the submucosa, areas of edema in the lungs, with subpleural hemorrhages

Heart's blood urea at necropsy, 1,680 mg

Bile urea at necropsy, 1,782 mg

April 19, 1920—Dog 3, brown female, 9 kg, received 115 gm urea (under ether anesthesia) and developed generalized tremors, respiratory paralysis and death within fifteen minutes after the injection Blood urea rose from 218 mg to 1,125.6 mg The necropsy findings were similar to those in Dog 2, but with hemorrhages in the stomach alone, and no visible changes in the liver Bile urea at necropsy was 1,560 mg per 100 cc, urine urea, 2,670 mg

June 14, 1920—Dog 10, brown female, 89 kg, received 120 gm urea Marked salivation, restlessness, tremors, tonic and clonic convulsions, opisthotonos, nystagmus, dyspnea, diarrhea, subnormal temperature, bloody urination and death resulted Blood urea rose from 242 mg to 1,644 mg Urine urea rose to 3,230 mg Necropsy showed the usual findings, very similar to those in Dogs 2 and 8 Heart's blood urea, 1,702 mg, bile urea, 1,728 mg

April 26, 1920—Dog 6, brown and black female, weighing 139 kg

4 10 p m—Temperature, 103.4 F

5 15 p m—Normal blood urea, 215 mg

5 40-6 30 p m—Injected 105 gm urea in toto

5 48 p m—After 15 gm, dyspnea (180 per minute), heart, 96

5 54 p m—After 30 gm, vomiting of yellow mucus, respiration, 30, heart, 96

6 03 p m—After 45 gm, visible peristalsis, temperature, 101.6 F

6 05 p m—After 50 gm, salivation of a thick, viscid, stringy, mucous saliva, with 412.8 mg urea

6 15 p m—After 70 gm, deep labored breathing (48 per minute), heart, 180, rapid horizontal nystagmus, salivation, marked prostration

6 18 p m—After 80 gm, vertical nystagmus, dyspnea, tremors of the limbs

6 23 p m—After 85 gm, slight tremors of the head and neck, tendency to opisthotonos

6 25 p m—After 90 gm, respiration, 144, dog almost chokes with thick mucus, stringy saliva pours from mouth

6 30 p m—After 105 gm, backward jerkings of the head

6 32 p m—Generalized clonic convulsions for one minute, animal in coma, corneal reflexes absent

6 34 p m—Clonic convulsions for a few seconds, then sudden cessation of heart beat and respiration, with death Temperature, 104.8 F

6 50 p m—Blood urea at necropsy 669.5 mg, urine urea, 3,690 mg per 100 cc, bile urea, 1,260 mg, gastric contents, 1,236 mg urea per 100 cc, intestinal contents, 734 mg urea (per 100 cc)

Necropsy showed some edema in the lungs, hemorrhages (submucous) in the stomach, hyperemia of brain and cord, the kidneys large, tense, bluish and dry on section, with grayish, cortical streaks

Histologic examination showed marked fatty degeneration in the cortical rays, with cloudy swelling of the convoluted tubules of the kidneys, slight fatty changes in the liver, and a patchy congestion and edema of the lungs

May 6, 1920—Dog 7, black female, weighing 16 kg

4 15 p m—Temperature, 104.8 F, urine, 2,470 mg urea per 100 c c, blood urea, 236 mg

4 50-5 52 p m—Injected 94 gm urea At 45 gm, nausea, salivation, dyspnea, at 45 gm, vomiting, at 68 gm, much vomiting, respiration, 20, heart, 72, temperature, 101.2 F

6 03-6 45 p m—Seventy-nine gm urea injected Total injected, 173 gm

6 10 p m—Temperature, 101 F, respiration, 24, heart, 66 At 136 gm, dog's mouth full of viscid, foamy saliva, jerky grunting respiration, at 170 gm, vomiting and several times thereafter

7 25 p m—Blood urea, 1,452 mg

7 30 p m—Temperature, 99 F

8 30 p m—Temperature, 95.4 F, dog restless, dyspneic, cannot stand, in stuporous state conscious only of strong stimuli, died between 9 p m and 8 a m, found very rigid

May 7, 1920, 11 a m—Necropsy showed hyperemia and hemorrhages (submucous) in the small intestine, pylorus, and the upper part of the colon, the left kidney large, flabby, cyanotic, with light gray streaks in the cortex, the right kidney half the size of the left, with knobby surface, depressed scars and adherent capsule, firm on cutting, with a large white scar in the pyramids and fibrous patches in the cortex, the heart with subendocardial hemorrhages in the right auricle, the lungs edematous, especially in the lower lobes and posteriorly, with bloody frothy fluid in all the bronchi, the brain and cord hyperemic and wet

Histologic examination showed marked fatty degeneration in the cortical rays with cloudy swelling of the convoluted tubules of the kidney, scars of chronic interstitial nephritis in one kidney with hyaline casts, hypostatic congestion and edema in the lungs

Blood urea at necropsy, 1,539 mg, urine urea, 3,870 mg per 100 c c, bile urea, 1,242 mg, intestinal contents, 1,836 mg urea

May 17, 1920—Dog 8, white female, weighing 11.5 kg

4 03 p m—Normal blood urea, 119 mg

4 03-5 08 p m—Injected 130 gm purified urea

[In order to eliminate ammonium salts as a possible cause of the symptoms, a quantity of urea (Merck's) was purified by recrystallization from alcohol, so that the solution of urea was practically neutral and less than 1 mg ammonia nitrogen per 10 gm urea could be obtained on aeration of a 33½ per cent urea solution Ordinary urea contained, on the average, 10 mg free ammonia nitrogen per 10 gm urea]

4 20 p m—Temperature, 102.6 F, heart rate, 96, respiration, 14 At 30 gm urea, salivation, at 60 gm urea, nystagmus, dog seems depressed

4 40 p m—At 75 gm urea, very thick stringy saliva, containing 474 mg urea, the animal's head was thrown back in opisthotonos

4 50 p m—Urea, 90 gm, slow backward jerkings of the head

4 53 p m—Urea, 97 gm, slight tremors of the head, marked opisthotonos and nystagmus

4 56 p m—Urea, 102 gm, slight generalized tremors, snapping movements of the jaws, temperature, 102.2 F

5 02 p m—Urea, 116 gm, marked generalized tremors, increased reflex irritability

5 04 p m—Urea, 120 gm, heart rate, 84, respiration, 20, shallow Coarse twitchings and marked opisthotonos

5 08 p m—Urea, 130 gm, dog released, tries to stand, but has clonic convulsions and falls to the floor

5 10 p m—Convulsions again, the animal rolls his head and body over and over, pants rapidly for one and one-half minutes, then tremors appear

5 12 p m—Convulsions again for one-half minute, heart rate 122, the animal goes into convulsions on being touched, convulsions and opisthotonos for three minutes following the insertion of a thermometer into the rectum, then sudden cessation of convulsions, with marked panting respirations and flaccid extension of the limbs

5 20 p m—Temperature, 105.2 F, respiration, 204, heart, 66 and weak

5 33 p m—Blood urea, 1,242 mg, dog breathing jerkily, in constant tremor and opisthotonos

5 40 p m—Temperature, 102 F

5 50 p m—Respiration, 78, heart, 66, temperature, 100.4 F, generalized tremors, corneal reflex now present

6 20 p m—Urinated 150 c.c. bloody urine, 3,730 mg urea per 100 c.c.

6 55 p m—Temperature, 98.4 F, respiration, 28, heart, 66, tremors

7 45 p m—Temperature, 98 F, no tremors, animal in stupor

The animal died between 8 p m and 8 a m, May 18, 1920

May 18, 1920, 1 p m—Necropsy showed the small intestine hyperemic, with marked hyperemia and submucous hemorrhages in the stomach, duodenum and upper jejunum, the urinary bladder empty, the kidneys bluish, with injected cortical vessels and grayish streaks in the boundary zone, the liver pale and grayish on section, the heart with subendocardial hemorrhages in the left ventricle, the lungs with marked congestion and edema, and extensive petechial and purpuric extravasations under the pleura, the brain and spinal cord hyperemic

Histologic examination showed very marked fatty degeneration in the cortical rays, with cloudy swelling of the convoluted tubules of the kidney, some peripheral fatty changes in the liver, hypostatic congestion and edema of the lungs

Heart's blood urea at necropsy, 1,155 mg, bile urea, 978 mg

May 20, 1920—Dog 9, brown female, 10.7 kg, with large goiter, received 118 gm repurified urea and developed nystagmus, generalized twitchings, clonic convulsions, opisthotonos, dyspnea, bloody diarrhea, stupor, subnormal temperature, death, very similar to the picture described in Dog 8. Blood urea rose from 20.2 to 1,776 mg. Urine urea was 3,010 mg at the end of the injection. Necropsy findings were similar to those in Dog 8, but with less marked lung involvement, fatty changes in the central parts of the liver lobules, and marked nodular and cystic goiter

Heart's blood urea, 1,362 mg, gastric contents' urea, 1,923 mg, bile urea 1,422 mg

May 16, 1920—Dog 5 black female, weighing 12.5 kg, normal blood urea, 18.5 mg. The right ureter was doubly ligated, under ether anesthesia. Blood urea after operation, 22.5 mg

May 24—Blood urea, 21.3 mg, the wound is superficially infected, the stitches loose, some were removed. The dog weighs 11.15 kg

May 30—Dog weighs 11.9 kg, wound granulating well. Blood urea 17.2 mg

4 30 p m—Respiration, 24, heart, 126, temperature, 103 F

4 40-5 25 p m—Urea, 87 gm (Daigger), injected

4 55 p m—At 30 gm, respiration, 24, heart, 104, temperature, 102.8 F

5 00 p m—At 40 gm, signs of nausea

5 07 p m—At 55 gm, ineffectual attempts at vomiting

5 15 p m—At 70 gm, respiration, 28, heart, 100, temperature, 102.8 F

5 18 p m—At 75 gm, tremors of the head and forelimbs, backward throwing of the head

5 25 p m—At 87 gm, sudden vomiting, with tonic and clonic convulsions, opisthotonos, nystagmus, urinated 75 c.c. pale cloudy urine, containing 1,690 mg urea per 100 c.c. The animal tries to get up, and keeps rolling his head over and over, paddling movements of the limbs

5 33 p m—Convulsions cease for a few seconds, but begin again, lasting two minutes

5 46 p m—Respiration stopped suddenly

5 48 p m—Heart stopped beating

6 15 p m—Necropsy showed the laparotomy wound to be clean on the peritoneal surface with a few adhesions, the distal end of the right (ligated) ureter was adherent to the small intestine, the proximal end dilated (1 cm) and the pelvis much distended with pale, yellowish fluid so that the right kidney is twice the normal size, showing hemorrhagic necrotic areas in the medulla. Many white streaks in the cortex extending to the capsule as small solid pin-head sized projections, capsule not adherent, the left kidney somewhat enlarged, cyanotic, with light lower zone in the cortex, the lungs entirely collapsed, a few hemorrhagic areas in the pylorus and duodenum, the liver dark and full of blood, the brain and cord hyperemic

Heart's blood urea, 978 mg, bile urea, 1,185 mg

Histologic examination showed fatty degeneration in the limbs of Henle's loops, cloudy swelling in the convoluted tubules of the left kidney, with the tubules dilated and containing protein material, hemorrhages and necrotic areas, and evidences of an ascending suppurative pyelonephritis in the right kidney, some fatty changes in the liver

May 27, 1920—Dog 4, white female, 128 kg, had the right ureter ligated. Blood urea rose from 208 to 238 mg,

June 3—Sixty-three gm urea were injected, causing nausea, generalized tremors, tonic and clonic convulsions, nystagmus, dyspnea, diarrhea and stupor, from which the animal recovered, markedly depressed. Blood urea rose from 278 to 1,110 mg. Urine urea was 1,900 mg at the end of the injection

June 4—The dog is depressed. Blood urea, 265.5 mg

June 10—Seventy-four gm urea were injected, causing a picture similar to that noted June 3, with, again, recovery of the animal. However, the convulsions were much milder than previously. Blood urea rose from 201 mg to 933 mg. Urine urea was 2,040 mg

June 11—The dog seems well. Blood urea, 106.8 mg

June 14—Blood urea, 13 mg

June 26—Blood urea, 24.7 mg. The left ureter was ligated, the bowel being perforated accidentally. Blood urea rose to 272.4 mg. The dog was much depressed and vomited frequently, dying on the third day. Necropsy showed a suppurative peritonitis, pyohydronephrosis on the right side, some hydronephrosis on the left side with necrosis of the kidney tissue, hypostatic congestion and edema of the lungs. Heart's blood urea, 356.7 mg, bile urea, 369.3 mg, gastric contents' urea 448.2 mg

July 26, 1920—Dog 14, brown female weighing 132 kg, blood urea, 18.8 gm. The left ureter was ligated. Blood urea after operation, 30.6 mg

4 41 p m, 2 10 p m—Blood urea, 19.9 mg, some wound infection, respiration, 150, temperature, 106.4 F

4 50 p m, 5 53 p m—Injected urea 75 gm

4 53 p m—At 30 gm, respiration, 36, heart 168, temperature, 105.8 F and nystagmus. At 56 gm, some nystagmus

4 56 p m—At 75 gm, respiration, 36, heart, 36, temperature, 104.8 F. Some of the jaws, tremor, head

5 02 p m—Urea—Injected 30 gm urea. Total injected, 105 gm

5 04 p m—Urea, increased irritability, nystagmus, contracted pupils, slight twitchings and marked twitchings of the head and neck, with opisthotonos

5 08 p m—Urea, 15 convulsions of the head, neck and forelimbs

5 10 p m—Convulsion tries to stand over, pants rapidly for one tonic convulsions

3 14 p m—Opisthotonos, paddling movements, clonic convulsions alternate flexion and retraction of the head

3 17 p m—Convulsions continue, the animal defecates

3 18 p m—Marked clonic convulsions, with opisthotonos rolling movements, dyspnea with puffing out of the cheeks, diarrhea—lasting for two minutes

3 26 p m—Paddling movements, opisthotonos

3 30 p m—The animal is much quieter, but still has coarse tremors of the head and neck, with occasional paddling movements

3 40 p m—Blood urea, 1,096 mg

3 50 p m—The animal is panting, has much thick, mucus saliva, marked tenesmus and diarrhea

8 00 p m—Animal is very much depressed and in a drowsy state

August 1, 9 00 a m—Dog found dead

Necropsy showed some omental adhesions around the wound, subserous hemorrhages in the small intestine, with submucous hemorrhages in the stomach and duodenum, and marked hyperemia and edema of the rugae of the colon the left kidney one and one-half times larger than the right, very soft with numerous clusters of yellowish pinpoint areas on the surface of the kidney, alternating with red raised areas, marked hemorrhages in the pyramids and cortex, with yellow streaks in the cortex and necrotic grayish areas in the medulla, the right kidney slightly enlarged, having a boiled appearance, with grayish streaks in the lower half of the cortex, the liver pale, with darker centers, the lungs collapsed and airless, with little fluid, not much subpleural hemorrhage, the brain and cord unchanged

Histologic examination showed, in one kidney, the usual cloudy swelling and fatty degeneration, in the other kidney, an ascending suppurative process with hemorrhagic infarcts, necrosis and hemorrhage in the medulla and masses of organisms, some fragmentation of the myocardium, necrosis of the mucosa of the colon, with marked submucous edema, subpleural hemorrhages in the lung Heart's blood urea, 1,224 mg, bile urea, 1,263 mg, gastric contents' urea, 1,863 mg

July 29, 1920—Dog 15, black and white female, 67 kg, had the right ureter ligated Blood urea rose from 232 mg before, to 291 mg after, operation

August 5—Blood urea, 306 mg, 75 gm urea were injected, producing tremors, tonic and clonic convulsions, dyspnea, nystagmus, depression and death within sixteen hours Blood urea rose to 1,275 mg, urine urea to 1,730 mg Necropsy showed the usual fatty changes in the kidneys, with necrosis of the convoluted tubules in one kidney, hemorrhages in the intestine, and some pulmonary congestion and edema Bile urea, 1,659 mg, gastric contents' urea 1,768 mg

June 23, 1920—Dog 11, white and brown female, 62 kg, had both ureters ligated and cut The dog lived less than thirty-six hours showing dyspnea and depression Blood urea rose from 226 mg to 123 mg Necropsy showed yellowish streaks and hemorrhagic areas in both kidneys, hemorrhages and a patch of bronchopneumonia in the lungs, microscopically, fatty changes in the liver and kidneys, with necrosis of the convoluted tubules Heart's blood urea 243 mg, urine (in pelvis) urea, 609 mg, bile urea, 273 mg, gastric contents' urea, 350 mg

June 30, 1920—Dog 12, white female, weighing 61 kg

7 30 p m—Blood urea, 295 mg Both ureters were ligated and cut, the dog vomited after the operation

9 30 p m—Blood urea, 326 mg

July 1, 2 30 p m—The animal seems to be in good condition Temperature 102.4 F Blood urea, 92 mg

July 2, 9 00 a m—The dog is in fair condition but eats very little Temperature 101.6 F Blood urea, 198.7 mg

July 3, 10 00 a m—The animal is depressed and drowsy Temperature, 100 F Blood urea, 234.7 mg

8 00 p m—The animal is very much depressed and drowsy Temperature, 99.6 F Blood urea, 366.9 mg

10 30 p m—The animal is lying down in the cage, apparently in very poor spirits

July 4, 9 a m—The dog is very depressed and unresponsive Temperature, 100 F Blood urea, 432.3 mg

July 5, 9 00 a m—The dog was found dead

Necropsy showed some stitch abscesses in the laparotomy wound, but with the peritoneal surfaces perfectly clean, both kidneys slightly enlarged, the capsules strip readily, the medulla pale, with the cortex showing grayish-yellow streaks in its lower half and, in one kidney, a few small hemorrhages, both kidneys of a brown color, the liver dark and bloody on section, with the centers of the lobules very dark in color, the stomach and the upper part of the small intestine with circumscribed areas of necrosis, the lungs collapsed anteriorly, with boggy purplish areas posteriorly and subpleural hemorrhages in some of the lobes, the brain and cord unchanged

Histologically, the kidneys showed granular degeneration and necrosis of the convoluted tubules, with abscesses in some areas, hemorrhage, albumin precipitate in the glomerular spaces, fatty degeneration of the cortical ray tubules, and dilatation of some of the convoluted tubules, the intestine and stomach showed areas of necrosis of the villi and glands, respectively, with some submucous edema, the liver showed some central congestion, while in the lungs, hypostatic congestion edema, and areas of subpleural hemorrhage were seen Heart's blood urea 329.1 mg, urine (from pelvis) urea 605 mg, gastric contents' urea, 606.9 mg, bile urea, 469.5 mg

July 7-8, 1920—Dog 13 yellow and white female, 6.5 kg, had both ureters ligated and cut The animal became much depressed and died within eighty-four hours Blood urea rose gradually from 26.9 mg to 415.5 mg Necropsy showed the usual fatty changes and hemorrhages in the kidneys some hemorrhage in the duodenum, congestion and edema of the lungs Heart's blood urea, 502.8 mg, bile urea, 538.5 mg, gastric contents' urea, 626 mg, urine (in pelvis) urea, 780.8 mg

SUMMARY

1 A series of three experiments was carried out on two dogs, demonstrating the rapid diffusion of urea into the tissues Hence, within only a few minutes after the injection of 20, 30, 40, or 80 gm urea, in divided doses of from 10 to 15 gm at a time, the blood urea content was found to be very much what might be expected from the relationship between the weight of the blood in the body and the weight of the body as a whole Thus, in a dog weighing 9.5 kg the injection of 30 gm urea gave a blood urea of 348.3 mg per 100 c c, in a dog weighing 16.3 kg, 20 gm urea gave 161.4 mg, 40 gm urea gave 327.3 mg, and 80 gm urea gave 491 mg urea per 100 c c With the injection of from 30 to 40 gm urea or more, salivation and vomiting were produced, but no other effects of any moment

2 A second series of five experiments on five dogs was carried out to determine the toxicity of urea The urea was injected practically continuously at the rate of 2 gm urea (in 33 1/3 per cent solution) per minute The average normal blood urea content of all the dogs used

was 207 mg per 100 c c—the extremes being 119 and 295 mg, respectively

In Dogs 2, 3, 6, 7 and 10, weighing 166, 90, 139, 160 and 89 kg, respectively, 2023, 115, 105, 173 and 120 gm urea, respectively, or, in other words, 12, 13, 075, 11 and 13 per cent, respectively, of the total body weight, an average of 11 per cent were required to produce toxic symptoms such as generalized tremors and convulsions, coma and death. Hence, in order to produce serious symptoms, leading to death, one must inject, intravenously, at least 1 per cent of the dog's weight in grams of urea. This confirms the results reported by Marshall and Davis⁴⁵

In this group of experiments there were rather broad variations in the symptoms produced, but certain symptoms were almost uniformly present. Among the earliest and most constant was salivation—of a thick, stringy, mucus type—appearing after from 25 to 50 gm urea had been injected, and with distinct evidence of excretion of urea in the saliva. Thus, in Dog 6, after 50 gm of urea had been injected, the thick saliva contained 4128 mg urea per 100 c c, in Dog 10, after injection of 56 gm urea, the saliva contained 4968 mg urea per 100 c c.

Vomiting was another constant symptom, usually coming on after 40 gm urea, or more, had been given. It varied considerably among the different dogs, and was usually productive of gastric contents and some bile tinged mucus. It is interesting to note that in the only case (Dog 7) in which no convulsions were seen, vomiting was a very prominent symptom, and in this dog was seen the only example of chronic nephritis, affecting only one kidney but with very marked fibrosis.

In all the dogs of this series, after a sufficient amount of urea had been injected, increased reflex irritability, with nystagmus, tremors beginning in the head and neck and spreading downward, coarse twitchings, opisthotonic retraction of the head, tonic then clonic convulsions (except in Dog 7), unconsciousness, marked dyspnea, coma, diarrhea, and a gradual fall in temperature and pulse rate appeared in the order given, and lasted from a few minutes to many hours, most of the animals dying within twelve hours or less after the injection, two (Dogs 3 and 6) dying suddenly from respiratory paralysis. Bloody urination was rather a constant feature, the urine containing from 2,670 to 5,090 mg urea per 100 c c. While the picture presented by the animals varied somewhat in the relative predominance of its various elements, it could not be mistaken for anything but urea intoxication. If, after hours of twitchings and irregular panting respiration, with deep stupor and profound prostration, the animal recovered consciousness, it was very much depressed, weak, almost unable to stand, and

would lie in the cage totally unresponsive to ordinary stimuli, as if in a deep sleep, yet conscious

The blood urea, determined about thirty minutes before or after the end of the injection, varied, of course, ranging from 669.5 mg (rather low) in Dog 6 to 1,812 mg in Dog 2, an average of 1,327 mg urea per 100 c c

The necropsy findings in this series were strikingly uniform, consisting chiefly of marked hemorrhages in the submucosa and mucosa of the stomach and small intestine, with necrosis of the mucosa in some areas, subpleural hemorrhages with varying amounts of hypostatic congestion and edema of the lungs, and very marked fatty degeneration of the limbs of Henle's loop, with cloudy swelling of the convoluted tubules of the kidney. Apparently, therefore, the urea acted as an endothelial cell poison, and also as a toxic agent for renal epithelium.

The heart's blood urea content, postmortem, varied from 669.5 mg to 1,702 mg per 100 c c, an average of 1,383 mg. The bile urea content varied from 1,242 mg to 1,782 mg, an average of 1,514 mg urea per 100 c c, indicating an excretion of the urea in the bile, as might be expected from its ready diffusibility.

3 The third series of experiments consisted of the determination of the lethal dose of repurified urea (by recrystallization from alcohol), in order to exclude the poisonous action of any foreign substance, such as ammonium salts. In Dogs 8 and 9, weighing 11.5 and 10.7 kg, respectively, 130 and 118 gm urea, respectively, were required to produce toxic symptoms, hence, 1.1 and 1.1 per cent of the body weight, just as with ordinary commercially pure urea. The symptoms in this series, as well as the blood, bile and urine findings, and the necropsy observations, were entirely similar to those presented in the preceding series, and need not be discussed further.

4 In the fourth series, a single ureter was ligated doubly, or ligated doubly and then sectioned between ligatures, in four dogs, the animals being allowed to recover largely from the operation and then their tolerance to urea injection estimated.

In Dogs 5, 4, 14 and 15, weighing 12.5, 10.5, 10.3, 13.2 and 6.7 kg, respectively, 87.0, 63, 105 and 75 gm urea were required to produce toxic symptoms, similar in every respect to those discussed above. Dog 4, curiously enough, received two separate injections, went into convulsions in each case, and recovered rapidly after both injections, although her symptoms were very much the same as those of the other dogs. In fact, this was the only instance of a dog recovering after convulsions. This animal was later operated on again and the remaining ureter ligated—as will be described under the next series.

It is seen, therefore, that in this series, with only one kidney functioning, the amount of urea necessary to produce toxic symptoms varied

from 0.6 to 1.1 per cent of the body weight, an average of 0.8 per cent, as compared with 1.1 per cent in animals with both kidneys functioning.

The general symptoms produced in this series were similar, in every respect, to those described in Series 2, and death occurred within about the same period of time after the injection. The blood urea content determined shortly before or after the end of the injection, varied from 933 to 1,275 mg, an average of 1,078 mg per 100 c.c. The bile and gastric contents urea showed distinct evidences of urea being excreted in those two fluids, particularly in the latter, in which the urea content averaged 1,815 mg per 100 c.c. in two dogs. The urine urea, after the injection, varied from 1,690 to 2,040 mg urea per 100 c.c.—distinctly low when compared with Series 2 and 3.

The necropsy findings in this series were, on the whole, quite similar to those in Series 2 and 3, except that the kidney corresponding to the ligated or divided ureter showed some hydronephrosis or pyonephrosis of a moderate degree, with necrosis of, and hemorrhages into the kidney tissue.

5 In the fifth and last series, four dogs were studied as to the effects of bilateral ligation and section of the ureters. These animals, Dogs 4, 11, 12 and 13, lived from three to four days, except Dog 11, which lived less than thirty-six hours. Unfortunately, not one of these animals was seen in the act of dying, so that while convulsions were never seen to occur, they may have taken place shortly before death. However, the absence of an early rigor mortis speaks against convulsions. In general, the symptoms were chiefly those of anorexia, a gradually falling temperature, an increasing drowsiness, and a marked depression, which was very obvious and constant. Irregularities in respiration appeared, but were not very constant. The blood urea rose steadily, reaching from 243 to 502.8 mg per 100 c.c., an average of 383.7 mg per 100 c.c. The bile urea varied from 273.0 to 538.5 mg per 100 c.c., an average of 412.6 mg per 100 c.c., the gastric contents urea ranged from 350.0 to 626.0 mg, an average of 507.8 mg per 100 c.c., indicating a distinct excretion of urea.

The necropsy findings in this series, of course, were those of degenerative changes in the kidneys, with a mild hydronephrosis, except in Dog 4, in whose right kidney (the right ureter was ligated long before the left) a very extreme hydronephrosis was present, also a suppurative peritonitis in the same animal. Dog 11 had a bronchopneumonic patch in one lung. The animals showed some hypostatic congestion and edema of the lungs, with a few subpleural hemorrhages. The stomach and intestines, in this series, were relatively unaffected, due, perhaps, to the low urea content of the blood as compared with that in the animals quoted in the second, third and fourth series.

CONCLUSIONS

The injection of urea, intravenously, in dogs produces a train of symptoms entirely analogous to that found in the convulsive or true uremia in man. Lesions are produced in the alimentary mucosa that may be related to uremic colitis. There seems to be a rather definite correlation between the severity of the symptoms and the concentration of urea in the blood.

Chronic uremia in man, ending ultimately in convulsions and coma, may be accounted for by urea intoxication, if we assume that the time element in the clinical cases is as important as the high concentration of urea in the animals, when injected. Obviously, more work is necessary, with particular reference to the use of such an apparatus as the Woodyatt machine for long continued injections of relatively low concentrations of urea. It is not implied here that only one factor is at play in the clinical forms of uremia, but urea retention must play a much more important rôle than that of an inert, harmless, waste product.

The asthenic condition produced by the ligation of both ureters involves probably many more factors than urea retention alone, and cannot, from the data at hand, be explained on any definite basis, except that such sudden occlusion of the ducts of one of the most important excretory systems must be followed by severe general metabolic disturbances.

Evidence was obtained that there is an active excretion of urea by the stomach, bile and intestine when excessive amounts are present in the blood.

I wish to thank Dr. Hanke of the Otho S. A. Sprague Memorial Institute for his helpful advice on several occasions and for purification of the commercial urea. I am deeply indebted to Prof. H. Gideon Wells for the suggestion of this problem and for his constructive criticism and cooperation throughout the entire time of this research.

METABOLIC STUDIES ON A CASE OF DIABETES INSIPIDUS *

I M RABINOWITCH, M D
MONTREAL, CANADA

Diabetes insipidus has been defined as a "chronic affection characterized by the passage of large quantities of normal urine of low specific gravity" ¹

The condition has been recognized for a long time, and the literature on the subject is large. Little, however, is found which bears on its study by the newer laboratory methods. Pathologists have furnished little gross or microscopic evidence as to the nature of this affection, except in those cases of the so-called "symptomatic type" in which an organic lesion in the brain has been found as a possible cause.

Experimentally, the possibility of a central origin for the condition has been suggested. Thus it has long been known that puncture of the floor of the fourth ventricle may produce a polyuria. No definite center for the control of polyuria, however, has been found.

Disturbances in the ductless glands have been suggested as the cause of this disease. These suggestions are based on the results obtained after the injection of extracts of the various glands. The evidence obtained is conflicting. The injection of pituitary extract has been shown to produce a polyuria, and cases in which a polyuria existed have been shown to improve after the administration of pituitary extract ². Although many experiments associate the pituitary body with a polyuria, such a specific action of this gland has been questioned. It has been shown ³ that a polyuria may be produced by a superficial lesion of the base of the brain, and this polyuria is greater than could be obtained after the injection of urea, caffeine or sodium chloride. A region has been localized (optopeduncular space) which seems to play some part in the mechanism of the regulation of water retention, with or without a polydipsia.

In 1914, Fitz, ⁴ in a review of the subject and the report of a case, summarizes the theories held as to the nature of diabetes insipidus as

* From the Department of Metabolism of the Montreal General Hospital.

1 Osler. Principles and Practice of Medicine, Ed 8, p 439.

2 Christie, C D, and Stewart, G N. Study of a Case of Diabetes Insipidus, with Special References to the Mechanism of Diuresis and the Action of Pituitary Extract on It, Arch Int Med **20** 10 (July) 1917. Schnabel T G, and Gerhard, A H. A Case of Diabetes Insipidus, New York M J **111** 812, 1920.

3 Camus, J, and Roussy, G. Experimental Researches on the Pituitary Body, Endocrinology **4** 507, 1920.

4 Fitz, R. Diabetes Insipidus, Arch Int Med **14** 706 (Nov) 1914.

follows (a) That it is due to the inability of the kidney to excrete a urine of normal concentration, (b) that it is due to hyperfunction of the pituitary gland with a resultant diuretic action, (c) that it is due to a primary polydipsia with a resultant polyuria

In the case reported by Fitz, the kidney function was found to be normal, except that it gave characteristic findings of a "vascular hypostenuria, with the vessels sensitive to chlorid stimulation"

More recently, emphasis has been placed on the tissues of the body as a factor in the production of polyuria. As a result of certain chemicophysical changes, osmotic pressure, etc., the tissues of certain organs are unable to retain their full quota of water, and polyuria results

In another theory an endeavor has been made to correlate a central origin with a renal factor,⁵ and on this basis diabetes insipidus is regarded as a disease produced by the insufficiency or lack of internal secretion of the pars intermedia of the hypophysis, a secretion which normally regulates diuresis, by acting on the renal cells, causing vasodilatation of the arteries of the kidney

The interesting problems involved, the numerous clinical methods now available for their study and the uncommon occurrence of diabetes insipidus in this hospital (the present case being the only one recorded in the last 50,000 admissions to the wards) have stimulated the metabolic study of the following case

REPORT OF CASE

A male (Hospital No 666-21), aged 47, was admitted Feb 17, 1921, to the medical wards of the Montreal General Hospital, service of Dr H A Lafleur, who made the clinical diagnosis. The patient complained of a craving for water and frequency of micturition

Personal History—Canadian farmer, married, no previous illness, except a radical mastoid operation in July, 1920, with, apparently complete recovery. Denies venereal disease

Family History—Irrelevant, no suggestion of syphilis

Present Illness—Apparently well until January, 1921, when he first noticed frequency of micturition and increasing thirst. The history is indefinite as to which of these appeared first. He consulted his physician, who asked him to measure his urine for twenty-four hours, when he found he voided seven quarts. Except for the large quantity, it was normal

Physical Examination—General examination negative, except for an old depressed scar at the site of a mastoid operation. Lungs negative

Circulatory System—Blood pressure systolic, 155, diastolic, 95, otherwise negative

Nervous System—All reflexes were normal, no evidence of intracranial pressure, except that the spinal fluid during lumbar puncture was under great pressure

CLINICAL LABORATORY REPORTS

Urine—Clear, pale, acid reaction, no sediment, sp gr, 1 002, no albumin, no sugar Microscopic examination negative

Blood—Red blood cells, 4,864,000, leukocytes, 9,000, hemoglobin, 100 per cent (Sahli) Differential count normal Wassermann negative

Spinal Fluid—Clear, under great pressure, cell count, 9 per c mm all globulin tests negative Wasserman negative

Röntgenogram of Skull—Shows no abnormality of the pituitary fossa, except that the fossa is shallow and the clinoid processes are indistinct Sphenoidal sinus clear

METABOLISM STUDIES

Since certain observations made are applicable to the study of more than one of the various problems which suggested themselves, and repetition of these observations was not practicable, they are recorded with comment only, but all are correlated under the general discussion

GENERAL BLOOD CHEMISTRY

Nonprotein nitrogen	28 0	mg per 100 c c
Urea nitrogen	16 0	mg per 100 c c
Uric acid	11	mg per 100 c c
Creatinin	12	mg per 100 c c
Cholesterol	0 126	mg per 100 c c
Sugar	0 100	per cent
Chlorids (as sodium chlorid)	0 627	per cent (plasma)
Total protein	7 4	per cent
Albumin	4 6	per cent
Globulin	2 8	per cent

The only abnormal finding here is the hyperchloremia

ENDOCRINE STUDIES

The close association of the function of the various endocrine organs has repeatedly been demonstrated Since certain of these, e g, suprarenal, thyroid and pituitary, have been shown to effect diuresis, these were studied

BASAL METABOLISM

Apparatus used Benedict respiration apparatus⁶

Method—The examinations were always made when the patient was in the postabsorptive state, and only after a preliminary rest period,⁷ when the pulse rate, blood pressure and respiration rate were determined The average normal oxygen consumption per minute, per square meter of body surface was taken from the tables of calories given by Drs Aub and DuBois⁸ using an average value of 4 83 calories per liter of oxygen, and an average normal respiratory quotient of 0 82 Under normal average conditions, 1 c c oxygen consumed has a value for heat production of 0 29 calories for each square meter of body

6 Benedict, F G A Portable Respiration Apparatus for Clinical Use, Boston M & S J 178 867, 1918

7 Boothby, W M, and Sandiford, I Technic of Basal Metabolic Rates Determinations, Philadelphia, W B Saunders Company, 1920

8 Aub, J C, and DuBois, E F The Basal Metabolism of Old Men, Arch Int Med 19 823 (May) 1917

surface The body surface was calculated from the formula of DuBois and DuBois⁹ $A-W 0.425 \times H 0.725 \times 7.25$ After the normal basal values of the patient were found, the effect was noted after the administration of epinephrin chlorid, a pituitary extract and thyroxin

During the period of this observation the blood pressure and pulse rate were taken every five minutes for two hours, and the basal metabolic rate was determined for a ten minute period at the end of 10, 30, 60, 90 and 120 minutes, respectively

TABLE 1—EPINEPHRIN CURVE

	Pulse per Minute	Blood Pressure		Respira- tions per Minute	Metabolic Rate	
		Systolic	Diastolic		O ₂ Oxygen per Min per Sq Meter	Percentage Devia- tion from Basal
					Body Surface	
Basal	72	150	96	24	134.3	
0.5 c.c. epinephrin, 1:1,000 solution						
10 minutes later	76	152	96	24	147.6	11
30 minutes later	72	150	96	24	144.9	9
60 minutes later	70	148	96	24	134.3	1
90 minutes later	61	150	96	24	135.6	2
120 minutes later	72	146	96	24	131.6	1

The thyroxin was given after the epinephrin and pituitary extract tests were completed

The normal findings under basal conditions do not justify the conclusion that there is no disturbance of the endocrine system, as it has been shown¹⁰ that the basal metabolism is the result of the sum total activities of the various glands. So long as one gland shows hyperfunction and another shows hypofunction to the same degree,

TABLE 2—METABOLIC RATE

	Metabolic Rate		Blood Pressure				Pulse		Respiration	
	Basal	High	Systolic		Diastolic		Basal	High	Basal	High
			Basal	High	Basal	High				
0.5 cc epinephrin 1:1,000	1	11	150	154	96	96	72	74	24	24
1 cc pituitary extract	2	4	156	164	92	92	74	80	24	24
10 mg thyroxin six days previously	2	18	148	160	92	86	70	88	24	28

the basal metabolism may be normal. The remarkable subnormal response to epinephrin in this case, as compared with the average findings reported in normal individuals,¹¹ suggests the possibility of

⁹ DuBois and DuBois, E. F. A Formula to Estimate the Approximate Surface Area if Height and Weight Be Known, *Arch Int Med* **17** 863 (June) 1916

¹⁰ Brock, S. R., and Kay, W. E. A Study of Unusual Endocrine Disturbances, *Arch Int Med* **27** 1 (Jan) 1921

¹¹ Sandiford, I. The Effect of the Subcutaneous Injection of Adrenalin Chlorid on the Heat Production, Blood Pressure and Pulse Rate in Man, *Am J Physiol* **51** 407, 1920

hypofunction of the chromaffin system. That this may play a part in the polyuria is possible, since it has been suggested¹² that the suprarenals act, in part, as a regulator of urinary excretion. To further investigate whether a hypofunction of the chromaffin system existed, the Csepar conjunctival,¹³ Goetsch subcutaneous epinephrin test, and Ascoli subepidermal epinephrin test¹⁴ were studied.

Csepar Conjunctival Test Normally three drops of 1:1000 epinephrin solution causes a slight or moderate blanching of the conjunctival sac persisting from ten to twenty minutes. In this case, the blanching was slight and persisted for six minutes. This test was regarded as negative.

The Goetsch test was negative (Table 3).

Ascoli Subepidermal Test Normally, 0.5 cc of 1:1,000 epinephrin solution causes the appearance of a swelling which, after a few seconds, assumes a dark inklike color. The swelling becomes surrounded

TABLE 3—RESPONSE TO GOETSCH EPINEPHRIN TEST
(5 cc epinephrin 1:1,000 solution)

	Pulse	Blood Pressure	
		Systolic	Diastolic
Before administration	72	152	88
2½ minutes later	72		
5 minutes later	74	150	90
7½ minutes later	72		
10 minutes later	72	150	90
12½ minutes later	68		
15 minutes later	72	148	86
17½ minutes later	68		
20 minutes later	68	150	88
22½ minutes later	68		
25 minutes later	72	152	88
27½ minutes later	70		
30 minutes later	68	150	90
60 minutes later	72		
90 minutes later	70	148	88

by a pale halo of irregular projections, surrounded by a red zone. Normally, this reaction may be found, according to Ascoli, with dilutions even of 1:200,000.

In the case studied, with a 1:1,000 solution the blue-black central spot was present, there was slight blanching, but no red halo, and, therefore, this test was only slightly positive.

Ascoli pointed out that in the great majority of normal individuals the pathologic reactions to epinephrin and pituitary extract are dissociated and opposed. Since diabetes insipidus has been associated

12 McLeod, J. J. R. *The Endocrine Organs, Physiology and Biochemistry in Modern Medicine*, Ed. 3, C. V. Mosby, St. Louis, p. 776, 1920.

13 Howard, C. P. *Functional Diagnosis of Polyglandular Disease*, etc., *Am. J. M. Sc.* **158**: 834, 1919.

14 Ascoli, M., and Faguoli, A. *Pituitrin Test*, *Endocrinology* **4**: 33, 1920.

with pituitary disease, tests for hypofunction of this gland were studied, including the Ascoli pituitary extract test¹⁴ and that of Brock and Kay¹⁰

Ascoli Test Using the usual strength of obstetric pituitary extract as sold in the ampules, a reaction similar to that of a solution of epinephrin 1 1,000 is supposed to occur. This, contrary to expectations, did not occur, except as a slight suggestion of redness

TABLE 4—RESULTS OF BROCK AND KAY'S TEST

	Pulse	Blood Pressure	
		Systolic	Diastolic
Before administration	74	136	92
1 cc of pituitary extract injected			
5 minutes later	84	154	90
10 minutes later	74	164	90
15 minutes later	72	156	92

Although there was a slight rise in the systolic pressure, it was not marked enough, taking the personal equation of reading into consideration, to regard it as definitely positive

Much clinical and experimental data tend to suggest that the pituitary body is concerned with carbohydrate metabolism. The finding of an increased sugar tolerance in acromegaly and pituitary tumors have been repeatedly reported. Keeton and Becht have shown that a hyperglycemia, associated with pituitary stimulation, is absent after transection of the spinal cord. Howard¹³ states that a diminished sugar tolerance in the presence of other symptoms, suggestive of disturbance of the pituitary body, justifies a diagnosis of increased activity of the pars intermedia.

TABLE 5—SUGAR TOLERANCE TEST

	Blood Sugar, per Cent	Urine Sugar
8 a m, fasting	0.125	0
100 gm glucose given		
9 a m	0.206	+
10 a m	0.168	+
11 a m	0.120	0
12 a m, 100 gm glucose given		
1 p m	0.196	+
2 p m	0.162	+
3 p m	0.126	0

Contradictory findings have recently been reported.⁷ Partial removal of one or both lobes of the pituitary body, or its total removal, did not appreciably modify the tolerance for carbohydrate, nor was it associated with the appearance of an alimentary glycosuria, and the injection of pituitary extract from the posterior lobe, anterior lobe or of the whole gland of the hypophysis did not sensibly modify—in the animals operated on—the tolerance for carbohydrate. Clinically,

however, the association of diseases of this gland and disturbance of sugar metabolism is definite. For this reason, the carbohydrate metabolism was studied. The method employed was the determination of the blood sugar, after a fifteen hour fasting period. Then 100 gm glucose in 250 c c water flavored with lemon juice was administered, and the degree of hyperglycemia and glycosuria was determined at one hour periods, until the original findings had returned. The method used in determining the blood sugar was that of Folin and Wu¹⁵

The blood sugar during the fasting period is rather high, with the method employed. Following the first administration of glucose, there is definite evidence of diminished tolerance, as shown by the glucosuria and suggestive evidence of delayed assimilation. The original concentration of the blood sugar was reached within the normal period, but the curve has a tendency to be rounded—not peaked. After the

TABLE 6—DATA ON URINE FOR TWELVE HOURS

Time	Intake	Output, C c	Specific Gravity
8 to 9 a m	450	100	1 004
9 to 10 a m	300	450	1 002
10 to 11 a m	310	250	1 003
11 a m to 12 m	220	90	1 004
12 m to 1 p m	150	145	1 006
1 to 2 p m	550	110	1 007
2 to 3 p m	150	100	1 006
3 to 4 p m	150	260	1 001
4 to 5 p m	320	270	1 001
5 to 6 p m	340	260	1 002
6 to 7 p m	360	560	1 002
7 to 8 p m	300	350	1 002
8 p m to 8 a m	0	1,600	1 001

second administration of glucose, there was also a diminished tolerance, and practically to the same degree. This makes the test more positive, since normally an increased tolerance is expected after repeated injections¹⁶

RESUMÉ—In the study of the basal metabolism of a case of diabetes insipidus a normal rate was found. There was no evidence of increased activity greater than would be expected with the dose of thyroxin administered. A diminished sugar tolerance was demonstrated, suggesting hyperfunction of the pituitary gland, and hypofunction of the suprarenals was suggested by subnormal response of the basal metabolic rate to epinephrin and negative Csepari, Goetsch and Ascoli tests. A balance, apparently, is struck between those two abnormal functions in keeping the basal metabolism unaltered.

15 Folin, O., and Wu Hsien. A System of Blood Analysis, Suppl. 1, J Biol Chem **41** 367, 1920

16 Hamman, L., and Hirschman, I. I. Studies on Blood Sugar, Bull Johns Hopkins Hosp **30** 306, 1919

DIETARY AND KIDNEY FUNCTION STUDIES

As a preliminary observation, the patient was allowed the regular full hospital diet and as much water as he cared to take. The intake of fluids was measured and the time at which they were consumed was noted. The urine was collected every hour, and the volume and specific gravity were recorded.

It will be noted that although, on the whole, there is some relation between intake and output of fluids, the lack of a definite relation occurred during the hourly periods, from 8 to 9 a m and from 1 to 2 p m, and again during the night when no fluid was taken. The persistent low specific gravity is marked. The total nitrogen for the day was 5 621 gm and for the night 7 302 gm, which is apparently the total average excretion on a full hospital diet. This figure has frequently been noted during preliminary observations in mild diabetics, in whom there was not an increased metabolism.

TABLE 7—RESULTS OF MOSENTHAL RENAL TEST MEAL

Hour	Urine		Sodium Chlorid		Nitrogen	
	C c	Specific Gravity	Gm	Per Cent Concentration	Gm	Per Cent Concentration
8 to 10 a m	90	1 020	0 108	0 120	0 921	1 02
10 a m to 12 m	100	1 016	0 160	0 160	1 008	1 00
12 m to 2 p m	135	1 013	0 432	0 320	1 285	0 95
2 to 4 p m	150	1 014	0 060	0 040	1 152	0 78
4 to 6 p m	115	1 015	0 092	0 080	1 255	1 09
6 to 8 p m	95	1 019	0 152	0 160	0 718	0 75
8 p m to 8 a m	390	1 020	0 780	0 200	5 460	1 04
	Balance		Intake		Output	
Water				1,760		1,075
Salt				8 5		1 184
Nitrogen				12 6		11 799

The marked nocturnal polyuria and low concentration of nitrogen (0 456 per cent) suggested impairment of kidney function, and seemed to support the theory that in diabetes insipidus, the kidneys are unable to excrete a concentrated urine. Therefore, the patient was placed on a Mosenthal renal test meal consisting of 12 6 gm nitrogen, 8 5 gm salt and 1,760 c c water, with the necessary restrictions. No food or fluids were allowed between meals. The meals were given at 8 a m, 12 noon and 5 p m, and the urine was collected at two hour periods from 8 a m to 8 p m and one specimen from 8 p m to 8 a m the following day. The possibility of the previous habits and diet influencing the test was excluded by giving the Mosenthal renal test for two days previous to the actual test.

The balance shows a slight water retention and more marked chlorid retention, and a normal nitrogen balance (allowing 10 per cent excretion by the feces). The night urine is normal in amount and in concentration of nitrogen, but low in salt. The two hour specimens

show a normal concentration of nitrogen, but are low in salt. With the exception of the salt metabolism, the findings are normal. A striking feature is the ability of the kidney to excrete a urine of higher specific gravity on a restricted fluid diet than on an unrestricted one, and yet in the preliminary observations, there seemed to be no definite relation between the intake and output of fluids.

Since the renal test meal seemed to indicate that the metabolism of salt and water was at fault, observations were made with special reference to these. The problem was whether the kidneys were unable to concentrate salt or whether there were certain physicochemical changes in the tissues which removed the salt from the blood, and the salt did not reach a high enough concentration (threshold) to be excreted. That is a study was made of the relation between the concentration of the chlorid in the blood and its rate of excretion as described by McLean¹⁷

The plasma chlorides were determined by the method of Whitehorn¹⁸. The normal threshold was accepted as 5.62 gm per liter.

$$\text{Calculated plasma NaCl} = 5.62 \sqrt{\frac{D \sqrt{C}}{W \times 4.23}}$$

$$\text{Calculated threshold} = \text{actual plasma NaCl} - \sqrt{\frac{D \sqrt{C}}{W \times 4.23}}$$

Where D — gm per 24 hours

Where C — gm per liter

Where W — weight in kilograms

The collection of specimens was followed as described by McLean¹⁹

	Plasma NaCl (gm per liter)		
	Actual	Calculated	Threshold
Determined under ordinary condition	6.281	5.641	6.260
10 gm NaCl	6.290	5.683	6.227
20 gm NaCl	6.273	6.002	5.891
10 gm NaCl + 10 gm urea	6.286	6.001	5.905
10 gm NaCl + 4 grams caffeine-sodium benzoate	6.286	5.679	6.227
10 gm NaCl + theobromin - sodium salt-cylate	6.261	5.682	6.201

RESUMÉ — Judging from the normal values obtained by this method, there was a persistent hyperchloremia and a persistent high threshold. The administration of sodium chlorid stimulated the kidney to activity by lowering the threshold, and this effect was greater with the increased dose of sodium chlorid. The administration of urea, together with the small dose of sodium chlorid, did have some effect in lowering the

17 McLean, F. C. The Numerical Laws Governing the Rate of Excretion of Urea and Chlorids in Man, *J. Exper. M.* **22**: 212, 1915.

18 Whitehorn, J. C. A System of Blood Analysis, Suppl. II, *J. Biol. Chem.* **45**: 449, 1921.

threshold, but not to the same degree as the administration of more salt. The diuretics, caffeine and theobromin, had no appreciable effect. The ability of the kidney to excrete nitrogen readily, as shown by the renal test meal, was corroborated by testing the effect on the blood and urine of the administration of urea.

The renal meal was repeated, but 10 gm urea were given by mouth at breakfast.

TABLE 8—RESULT OF SECOND RENAL TEST MEAL

Hour	Urine		Sodium Chlorid		Nitrogen	
	C c	Specific Gravity	Gm	Per Cent Concentration	Gm	Per Cent Concentration
8 to 10 a m	190	1.014	0.760	0.400	2.390	1.25
10 a m to 12 m	215	1.012	0.860	0.400	1.806	0.84
12 m to 2 p m	165	1.014	0.528	0.320	1.755	1.06
2 to 4 p m	160	1.015	0.896	0.540	1.747	1.09
4 to 6 p m	100	1.017	0.320	0.320	1.232	1.28
6 to 8 p m	90	1.019	0.216	0.240	1.285	1.42
8 p m to 5 a m	320	1.021	0.512	0.160	5.486	1.65
	1,240		4.092		15.501	

The balance shows that following the administration by mouth of urea there was a lessened tendency to retain salt and water and the nitrogen excretion was normal (allowing 10 per cent excretion by stools and urea = $2.14 \times \text{Urea N}$).

The ability of the kidney to excrete nitrogen is well shown in Table 9. Although sufficient urea was given to raise the blood urea nitrogen to about 200 mg per 100 c c (accepting 5 liters of blood as the average normal volume), the concentration of the blood urea nitrogen remained remarkably constant.

TABLE 9—BLOOD UREA

	Blood Urea Nitrogen, Mg per 100 C c
8 a m, before administration of 10 gm urea (orally)	16.1
9 a m	18.2
10 a m	16.3
11 a m	17.4
12 m	15.2

RESUME—From these findings the conclusion is justified that there is an inability on the part of the kidney to excrete chlorids properly. That the kidney is unable to concentrate all solids is not proven since its ability to concentrate nitrogen is remarkably good. The hyperchloremia, as found during the preliminary observations of this patient, is probably compensatory in nature, and favors excretion of sodium chlorid and is not a true retention. Clinically, there was no evidence of increase in weight, edema, etc. Since both a renal and hypophyseal factor are suggested in the case, observations were made on the effect of the administration of pituitary extract on the renal function.

The renal meal was then repeated, and 1 c c of a pituitary extract was administered hypodermically at the 8 a m, 12 m and 5 p m meals

TABLE 9—RESULT OF ADMINISTRATION OF PITUITARY EXTRACT

Hour	C c	Specific Gravity	Sodium Chlorid	
			Gm	Per Cent
8 to 10 a m	70	1 024	0 220	0 31
10 a m to 12 m	130	1 017	0 410	0 31
12 m to 2 p m	70	1 022	0 126	0 18
2 to 4 p m	150	1 020	0 510	0 34
4 to 6 p m	130	1 026	0 426	0 32
6 to 8 p m	100	1 020	0 316	0 316
8 p m to 8 a m	300	1 022	3 100	1 03
	950		5 108	

The injection of the pituitary extract was followed by a diminished diuresis, a retention of water and an increase in the salt excretion and concentration. That the concentration of salt is due to increased kidney activity and not only to retention of water is suggested by the greater excretion of salt and the analysis of the blood following the injection.

TABLE 10—SODIUM CHLORID IN BLOOD

Actual, Gm per Liter 6 002	Calculated, Gm per Liter 5 689	Threshold, Gm per Liter 5 933
-------------------------------	-----------------------------------	----------------------------------

It will be seen (Table 10) that the hyperchloremia has diminished, but in spite of this, excretion takes place at a lower threshold.

The effect of the pituitary extract soon wore off as shown in Table 11.

TABLE 11—INTAKE AND OUTPUT OF FLUID TWO DAYS LATER

Time	Intake	Output, C c	Specific Gravity
8 a m, breakfast			
8 to 9 a m	360	150	1 005
9 to 10 a m	260	400	1 001
10 to 11 a m	550	150	1 002
11 a m to 12 m	320	360	1 001
12 m to 1 p m	160	400	1 001
1 to 2 p m	260	265	1 003
2 to 3 p m	365	160	1 004
3 to 4 p m	410	175	1 003
4 to 5 p m	400	200	1 004
5 to 6 p m	150	260	1 005
6 to 7 p m	160	150	1 005
7 to 8 p m	280	200	1 006
8 p m to 8 a m	0	1,430	1 001

RÉSUMÉ—In the case of diabetes insipidus studied, no single specific lesion has been found to account for the polyuria.

Functional analysis of the endocrine system shows hyperfunction of the pituitary body and hypofunction of the suprarenals. A balance is struck between these two abnormal endocrine functions as shown

by the normal basal metabolism. Although the polyuria disappears on a restricted fluid intake, there is no evidence that the polyuria is due to a primary polydipsia. The kidney function is normal in every respect, except in its ability to concentrate salt. That the condition is due to physicochemical processes removing the salts from the blood and thereby not allowing a normal threshold to exist is not proven. The hyperchloremia found is apparently compensatory, aiding the excretion of salt and is not a true retention since there is no clinical evidence of salt retention. The administration of pituitary extract definitely caused a diminution of the polyuria and an increased rate of flow and an increased concentration of salt in the urine.

CONCLUSIONS

In a case of diabetes insipidus studied there is no one specific cause for the polyuria. An endocrine and a renal factor were found. Since the administration of pituitary extract improved not only the concentration but also the rate of excretion, it is suggested that the theory advanced that diabetes insipidus is produced by a lack of some internal secretion which normally regulates and moderates diuresis by acting on the renal cells holds in this case.

Archives of Internal Medicine

VOL 28

OCTOBER, 1921

No 4

STUDIES IN OXYGEN THERAPY WITH DETERMINATIONS OF THE BLOOD GASES

I IN CARDIAC INSUFFICIENCY AND RELATED CONDITIONS¹

ALVAN L BARACH, M D
AND
MARGARET N WOODWELL, A B
BOSTON

TABLE OF CONTENTS

- 1 Harmful effects produced by an insufficient oxygen supply
- 2 Evidence that an insufficient oxygen supply exists in clinical disease
- 3 Desirability of raising oxygen content of blood to normal level
- 4 Methods of administration in recent war
- 5 Method developed in this investigation
- 6 Illustrative cases of cardiac disease, bronchitis, edema of the lungs (Pneumonia, edema of the lungs, empyema and respiratory paralysis considered in Papers II and III)
- 7 Increase in oxygen content of blood following oxygen inhalation.
- 8 Clinical improvement that occurs in acute anoxemia, following oxygen inhalation

INTRODUCTION

The purpose of this investigation was primarily to determine what conditions of oxygen want could be relieved by the therapeutic inhalation of oxygen. The degree of anoxemia was determined by the oxygen saturation of the arterial and venous blood. The effect of oxygen administration was thus quantitatively measured by comparison of values before and after treatment. The correlation of the oxygen changes in the blood with the objective and subjective changes in the patient gave the opportunity of determining whether clinical improvement resulted from oxygen therapy. There were considered in all

¹From the Medical Service of the Massachusetts General Hospital. This paper is No 15 of a series of studies of the physiology and pathology of the blood from the Harvard Medical School and allied hospitals.

*The expense of this work was in part defrayed by a grant from the Proctor Fund of Harvard University for the study of chronic diseases.

two normal subjects, seven patients with cardiac insufficiency, twelve patients with pneumonia, and two patients with lethargic encephalitis. As the cases had to be studied individually, it became advisable for the sake of clearness to divide them into a cardiac group (Paper I), a pneumonia group (Paper II), and a group characterized by a type of shallow breathing in lethargic encephalitis (Paper III). An outcome of the work has been the development of an effective and practicable method of providing oxygen.

HISTORICAL

A few facts will be reviewed from the literature with the purpose of presenting a theoretical basis for the giving of oxygen. Barcroft¹ has shown from his own work and the work of others that any activity of the body organs involves a call for oxygen. Exact measurements have been made for the heart, kidney, muscle and the secreting glands which show that increased work is done only with a proportional increase in oxygen consumption. Stoppage of the oxygen supply brings the perfused heart to a standstill, causes a cessation in the flow of urine, decreases the conduction of nerve tissue, produces muscular fatigue, and at last immobility. Bayliss,² after reviewing the oxygen consumption of resting and active tissues, concluded that "an organ may suffer from oxygen want, even when the venous blood coming from it still contains oxygen, so that the oxygen passes the cells unused. It is probable that for proper functional work, oxygen must be supplied not only in certain amount, but at a tension not far below that in which it is present in the atmosphere and in the arterial blood."

The direct effects of anoxemia, as recently summarized by Haldane,³ have been obtained by observations at high altitudes, by laboratory experiments in which a lowered partial pressure of oxygen is breathed in chambers, and by the consequences of carbon monoxide poisoning, where the oxygen carrying power of hemoglobin is partly destroyed. One of the first symptoms of oxygen want, when gradually produced, is periodic breathing. At high altitudes, this is practically a normal phenomenon. The further symptoms include distinct impairment of mental faculties, often showing itself in manifestations similar to alcoholic intoxication. The powers of judgment, self control, and memory are definitely impaired. After some hours, there are nausea and headache, and often vomiting and diarrhea. If the exposure has been very long, though slight, these symptoms may still persist, even if meanwhile their cause has been removed. When lack of

1 Barcroft, J. *The Respiratory Function of the Blood*, 1914, p. 105.

2 Bayliss, W. M. *Principles of General Physiology*, 1918, p. 343.

3 Haldane, J. S. *Recent Developments in the Therapeutical Use of Oxygen*. Contributions to Medical and Biological Research Dedicated to Sir William Osler 1 550, 1919.

oxygen has been more serious, the pulse becomes rapid and feeble, periodic respiration gives place to rapid, shallow respiration, consciousness becomes greatly impaired or lost, and progressive damage is done to central nervous system, heart and other organs. The evidence is conclusive, Haldane believes, that these characteristic effects are due solely and simply to lack of oxygen.

The existence of cyanosis is *prima facie* evidence of lack of oxygen in clinical disease. Abnormal oxygen unsaturation of the venous blood in cardiac disease was found by Means and Newburgh.⁴ Lundsgaard⁵ later established the normal values of oxygen saturation of venous blood, and showed in various types of cardiac insufficiency abnormal oxygen unsaturation. The introduction of the arterial puncture by Hurter,⁶ and its successful development by Stadie,⁷ made the arterial blood of human beings accessible for study for the first time. Diminished arterial oxygen saturation was then shown to occur in pneumonia by Stadie,⁷ and in cardiac insufficiency by Harrop.⁸ In these investigations, the close relationship between cyanosis and the amount of reduced hemoglobin in the blood was established. The oxygen saturation of normal resting individuals may be taken as 95 to 98 per cent for the arterial blood, and 65 to 75 per cent for the venous blood.

Recently, Barcroft⁹ lived for six days in an atmosphere in which the partial pressure of oxygen fell to 84 mm. On the last day, the oxygen saturation of his arterial blood was 88 per cent, and after the performance of work 83.8 per cent. He lay in the chamber racked with headache, with occasional vomiting, and at times able to see clearly only as an effort of concentration. He became faint on exertion. His pulse, normally 56, had risen to 86. These effects were apparently due purely to oxygen want. The degree of anoxemia that produced them has frequently been found in pneumonia and heart disease by the investigators mentioned above. In many instances, the saturation of the arterial blood falls to far lower levels. It would, therefore, seem likely that lack of oxygen in the degree often found in disease would produce bodily discomfort, disturbances in function and damage to living structure.

4 Means, J. H., and Newburgh, L. H. Studies on the Blood Flow by the Method of Krogh and Lindhard. *Tr. Assn. Am. Phys.*, 1915, p. 51.

5 Lundsgaard, C. Studies of Oxygen in the Venous Blood, *J. Exper. M.* **27** 179, 1918.

6 Hurter. *Deutsch. Arch. f. klin. Med.* **108** 1, 1912.

7 Stadie, W. C. The Oxygen of the Arterial and Venous Blood in Pneumonia and Its Relation to Cyanosis, *J. Exper. M.* **30** 215, 1919.

8 Harrop, G. A. The Oxygen and Carbon Dioxid Content of Arterial and Venous Blood in Normal Individuals and in Patients with Anemia and Heart Disease, *J. Exper. M.* **30** 241, 1919.

9 Barcroft, J., Cook, A., Hartridge, H., Parsons, T. R., and Parsons, W. Flow of Oxygen Through the Pulmonary Epithelium, *J. Physiol.* **53** 450, 1920.

As a further consideration, there sometimes is an increased metabolic demand for oxygen in the diseases in which anoxemia is itself conspicuous. In cardiac insufficiency, an increased basal metabolism has been demonstrated.¹⁰ Fever causes an increased metabolism which has been proved to be proportional to the increase in fever.¹¹ It is of interest in this connection that the performance of physical exercise in normal men can be done easier, with less dyspnea and less fatigue, when oxygen is added to the inspired air.¹² This has been shown to be especially true of unfit men.¹³ Since oxygen helps the accomplishment of physical work in normal men, it might be expected to aid in the performance of the increased work in cardiac insufficiency and pneumonia. The above favorable results in the absence of anoxemia must depend on the increase of oxygen in physical solution.

We may refer briefly to certain recent methods of giving oxygen. The frequent occurrence of gas poisoning during the war led to a hurried demand for oxygen therapy. The condition produced by the irritant gases was an acute primary edema of the lungs characterized clinically by air-hunger and an extreme degree of cyanosis. Haldane¹⁴ devised an apparatus by which measured quantities of oxygen could be added to the inspired air, thus greatly increasing the available diffusion pressure through the alveolar wall. The essentials were an oxygen tank, a reducing valve, and a face mask. The mask was connected with a collecting bag which received oxygen from the tank, and with the outside air, from which the patient breathed. Oxygen was added to the inspired air in amounts of from one to four liters per minute. This was largely used in the acute cases with generally good results. Douglas¹⁵ found it very effective. Less effective was the nasal tube method, and still less effective and even more wasteful was the tube and funnel method. In the funnel method, Meltzer¹⁶ has calculated that the atmospheric air is not richer by more than 2 per cent, if by so much. Cummins¹⁷ found it difficult to make the

10 Peabody, F. W., Meyer, A. L., and Du Bois, E. F. Basal Metabolism of Patients with Cardiac and Renal Disease, *Arch Int Med* **17** 980 (July) 1917.

11 Coleman, W., and Du Bois, E. F. The Influence of the High Calory Diet on the Respiratory Exchanges in Typhoid Fever, *Arch Int Med* **14** 168, 1914. Also Du Bois, E. F. Respiration Calorimeter in Clinical Medicine, *Am J M Sc* **178** 792, 1916.

12 Hill, L., and Flack, M. Influence of Oxygen on Athletes, *J Physiol* **38** 12, 1909.

13 Briggs, H. Fitness and Breathing, *J Physiol* **54** 292, 1920.

14 Haldane, J. S. Therapeutic Administration of Oxygen, *Brit M J* **1** 181, 1917.

15 Douglas, C. G. Discussion on Therapeutic Uses of Oxygen, *Proc Roy Soc Med (Sec Therap & Pharmacol)* **13** 59, 1920.

16 Meltzer, S. J. Therapeutic Value of Oral Rhythmic Insufflation of Oxygen, *J A M A* **69** 1150 (Oct 6) 1917.

17 Cummins. Discussion on Therapeutic Uses of Oxygen, *Proc Roy Soc Med (Sec Therap & Pharmacol)* **13** 59 1920.

men stand the mask of the Haldane apparatus Ryle¹⁸ used the nasal catheter, introduced by Stokes, with fairly good results Masks were improvised at his station, but were not tolerated by the men Hamil¹⁹ considered the nasal tube satisfactory, but wasteful, and also found the mask badly tolerated, especially in warm climates Hoover²⁰ said the gassed men begged to be relieved of the mask and preferred to have the oxygen conducted by a soft rubber tube in their nostrils, though by the latter method he did not see a single case in which the cyanosis was diminished

The chronic cases of gas poisoning were treated in oxygen chambers at home, with striking evidences of improvement The group described by Barcroft, Hunt and Dufton²¹ exhibited disorder action of the heart, nocturnal dyspnea, high red blood cell counts, low percentages of carbon dioxide in the alveolar air, acidosis and kindred conditions The patients were kept in a chamber of 50 per cent oxygen for sixteen out of the twenty-four hours for five days Almost all of them showed a disappearance of symptoms Shufflebotham and Lowry²² treated cases of chronic gas poisoning in oxygen chambers with equally marked improvement They also treated one case of lobar pneumonia and one case of bronchopneumonia with promising results Meakins²³ treated a case of chronic gas poisoning with the Haldane apparatus, giving oxygen for three one-hour periods daily for seven days The patient became cured of all symptoms The arterial oxygen saturation was normal at the beginning, and increased by oxygen He also reported three cases of pneumonia and five cases of asthma, emphysema and chronic bronchitis, in which the arterial saturation was elevated by oxygen administration, and in whom marked improvement resulted No determinations of the venous blood were done, nor were any cases of cardiac insufficiency studied For patients who will not, or cannot, put up with a mask, Hill²⁴ constructed a simple oxygen bed tent, which encloses the upper part of the patient when in bed Arrangements are made for inlet and outlet of air and oxygen, for absorption

18 Ryle Ibid

19 Hamil Ibid

20 Hoover, C F Oxygen Therapy, J A M A **71** 880 (Sept 14) 1918

21 Barcroft, J, Hunt, G H, and Dufton, D The Treatment of Chronic Cases of Gas Poisoning by Continuous Oxygen Administration in Chambers, Quart J M **13** 179, 1920

22 Shufflebotham, F, and Lowry, G H Discussion on Therapeutic Uses of Oxygen, Proc Roy Soc Med **13** 59, 1920

23 Meakins, J C Observations on the Gases in Human Arterial Blood in Certain Pathological Pulmonary Conditions, and Their Treatment with Oxygen, J Path & Bact **24** 87, 1921

24 Hill, L A Simple Oxygen Bed Tent and Its Use in a Case of Edema and Chronic Ulcer of the Leg, J Physiol **50** (May 24) 1921, Proc Physiol Soc, p 20

of carbon dioxide, and for circulation of the air. A window of non-inflammable celluloid is placed in front of the patient. A soldier was treated who had had his femoral artery tied, and suffered from coldness, blueness, edema and chronic ulcers of the leg. In forty-eight hours, the oxygen made the extremity warm and of good color, removed the edema, cleaned up the ulcers, and started the healing in a remarkable way.

METHODS

The methods used in this investigation for giving oxygen have represented a gradual development in the apparatus used and in the duration of treatment. With the purpose first of determining to what extent and for what length of time oxygen could be increased in the blood, short periods of administration were given. Later, prolonged and repeated administration of oxygen was given with more of a therapeutic object. The first apparatus consisted simply of a face mask, connected through a soda-lime canister with a rebreathing bag, in turn connected with an oxygen tank. In this way, the patient rebreathed pure oxygen, the carbon dioxide being absorbed by soda-lime. This was soon modified by the insertion in the mask of a small inlet and outlet valve connected with the outside air. This provided the patient with about an 80 per cent oxygen mixture. All the connections were made one inch wide so that no resistance was felt on respiration. This was efficient, easily put together, and economical in the expenditure of oxygen. Its disadvantage was the use of the mask. There were a few patients who took it without objection and in comparative comfort. The greater number could not bear the suffocating feeling which they said it produced, and struggled against it. Especially when the patient was suffering with urgent dyspnea, or in warm weather, the mask was poorly tolerated. Later, when the Haldane apparatus was used, the same difficulty was experienced. When the patient was in coma the Haldane apparatus was very useful, for it was convenient to adjust the inlet of oxygen to the rate desired and leave it on for long periods. For the patients who would not submit to oxygen administration by a mask of any sort, a soft rubber mouthpiece such as is used in the Benedict Respiration apparatus was tried in its place with the rest of the rebreathing outfit unchanged. This proved to be very satisfactory. It fits easily into the mouth, its opening is wide enough for all respiration to take place through it, and its flanged margins support it and prevent leaking. When held or attached so that no pressure is brought to bear on the patient's mouth, it is comfortable, and causes nothing in the way of suffocation. When the nose is held, pure oxygen is rebreathed. This diminishes comfort, and was found to be unnecessary and undesirable, since patients get a rich oxygen mixture even when breathing through nose and mouth. The patient is asked

to breathe through his mouth, and usually adapts himself easily to the mouthpiece, breathing from four to seven parts oxygen through his mouth, and from three to six parts air through his nose. In the pneumonia patient, breathing takes place through mouth and nose even more naturally, and a mixture of about 50 per cent oxygen is usually inhaled. By watching the excursion of the rebreathing bag, the percentage of oxygen being breathed can be roughly estimated. The nose is held closed for a few inspirations, making all respiration take place through the mouth. When the nose is released, the diminution in the excursion of the bag indicates the proportion of breathing through the nose. This is, of course, only approximate, but there is no need for knowing the precise composition of the mixture. (Where this is desired, a Douglas bag can previously be filled with a known mixture of oxygen, and attached in place of the tank.) There seem to be two important considerations in giving oxygen: one, that an effective oxygen mixture be used, two, that pure oxygen should not be given for long periods. Lorrain Smith²⁵ was able to produce pneumonia in animals by having them live in atmospheres of pure oxygen for several days. Karsner²⁶ also produced pneumonia in rabbits by having them breathe from 80 to 90 per cent oxygen in chambers for two or more days, but was able to demonstrate no noteworthy changes in other organs. Mixtures under 70 per cent oxygen can be breathed indefinitely without harmful effect²⁷. That no increase in the vital process occurs during the inhalation of pure oxygen was first demonstrated by Lavoisier and Seguin²⁸ in 1789, and after a century of dispute was finally carefully confirmed by Benedict and Higgins²⁹.

There are patients who will for a while inhale through the mouth and exhale through the nose or inhale through the nose and exhale through the mouth, in the first case not rebreathing and consequently wasting the oxygen, and in the second place distending the rebreathing bag. This is usually remedied by instruction. There are, of course, patients in active delirium who will not put up with mask or mouthpiece. As a rule, however, the patient takes the mouthpiece well, and breathes a satisfactory mixture. No matter how ill, it involves no actual burden, patients who struggled against the mask frequently took the mouthpiece quietly and even dozed off during the administration. (See illustration for arrangement of apparatus.)

25 Smith, J. L. The Pathological Effects Due to Increase of Oxygen Tension in the Air Breathed, *J. Physiol.* **24** 19, 1899.

26 Karsner, H. T. The Pathological Effects of Atmospheres Rich in Oxygen, *J. Exper. M.* **23** 149, 1916.

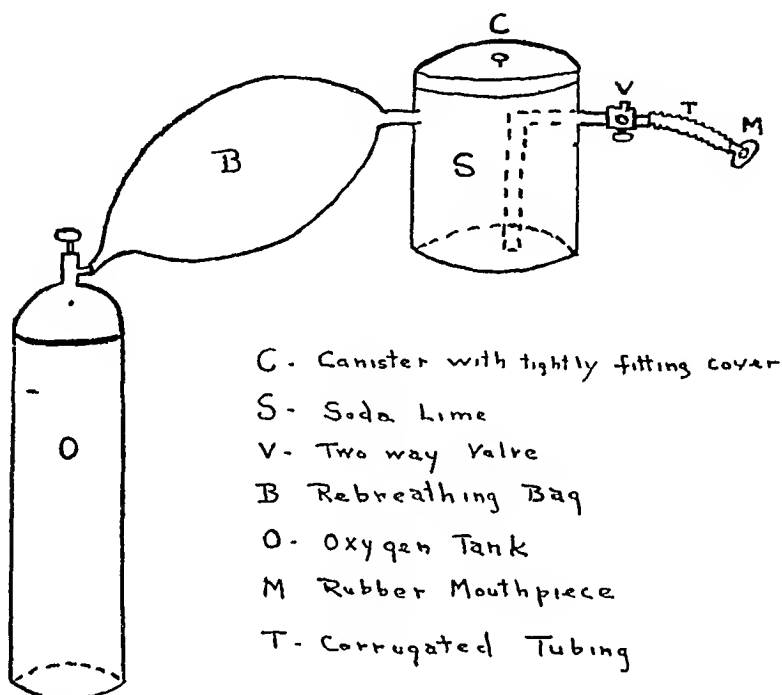
27 Flack, M., and Hill, L. *Textbook of Physiology*, 1919, p. 303.

28 Lavoisier and Seguin. *Mém. de l'Acad. des Sc.*, 1789, p. 185.

29 Benedict, F. G., and Higgins, H. L. Effects on Men at rest of Breathing Oxygen-Rich Gas Mixtures, *Am. J. Physiol.* **28** 1, 1916.

The apparatus is easily put together and carried about, and is not wasteful in its consumption of oxygen (The duration and efficiency of the soda-lime can be tested by running the air in the rebreathing bag through a barium hydrate solution)

The gas analysis, except for the instances mentioned below, was done by Van Slyke and Stadie's ³⁰ method of determining oxygen and carbon dioxide on the same sample of blood. A modified Van Slyke vacuum apparatus is used, in which the pipet is twice the length and one-half the diameter of the original one, and calibrated to 0.01 c.c. The Van Slyke ³¹ method of determining oxygen alone (with the recent correc-



Apparatus for giving oxygen The patient breathes through the rubber mouthpiece M through the can of soda-lime C into a rebreathing bag B. The carbon dioxide exhaled is removed by the soda-lime, and oxygen is admitted from the tank O at a sufficient rate to keep B inflated. In this way the patient rebreathes pure oxygen from the apparatus, but since his nose is left open he dilutes this with a certain proportion of atmospheric air. In practice this results in the inhalation of from 40 to 60 per cent oxygen.

tions for dissolved gases) was used in Case 4, the analyses in Case 3 that were done Oct 16 and Oct 20, 1921, and the analyses in Case 2 that were done Oct 6, 1921. The arterial and venous blood was drawn without stasis under oil after the method described by Stadie.⁷ The analyses were done in duplicate, in most cases directly after the blood was taken. When the blood was allowed to stand from one to three

³⁰ Van Slyke, D. D., and Stadie, W. C. J. Biol. Chem., To be published

³¹ Van Slyke, D. D. Gasometric Determination of the Oxygen and Hemoglobin of Blood, J. Biol. Chem., **33** 127, 1918

hours it was kept on ice. Lundsgaard found that blood under oil on ice suffered no change in oxygen content in twenty-four hours. Control determinations done by ourselves at these varying intervals showed no significant change in gas content.

PRESENTATION OF DATA

It will help in the consideration of our cases if the several types of anoxemia are borne in mind. According to the classification of Barcroft,³² they are as follows:

1 *Anoxic Type*—The pressure of the arterial blood is too low. The arterial oxygen saturation of the hemoglobin is below normal, i. e., below 95 per cent. Mountain sickness is an example in which the diminished partial pressure of oxygen in the blood is due to the diminished partial pressure of oxygen in the atmosphere. In edema of the lungs, the oxygen at prevailing pressures cannot diffuse properly through the diseased alveolar wall, and some unexpired blood is passed into the aortic stream, lowering the arterial saturation.

2 *Stagnant Type*—The quantity of oxygen which reaches the tissues in unit of time is less than normal because of a slowed blood flow. More oxygen is used up in the capillaries and the venous blood has a lowered oxygen saturation, below 65 per cent.

3 *Anemic Type*—A diminished amount of available hemoglobin is present, which involves a lessened capacity to carry oxygen. This condition may be due to an actual diminution in the number of red blood cells, or the hemoglobin may be converted into methemoglobin, or monopolized by carbon monoxide so that its oxygen carrying function is destroyed.

The anoxic type is primarily due to pulmonary causes, i. e., faulty oxygenation of blood as it flows through the lungs. The stagnant type is primarily due to cardiac causes, i. e., decreased rate of blood through the systemic capillaries. In cardiac insufficiency, the two frequently occur together, the stagnant type because of poor heart action, the anoxic type because of the secondary conditions of pulmonary congestion and edema.

PROTOCOL OF EXPERIMENTS

The individual cases follow, the first two being normal controls.

CASE 1 (Table 1)—Normal. White female, research worker, age, 23 years.

EXPERIMENT—Nov. 18, 1920. Subject in recumbent position, three hours after breakfast. Pure oxygen was given through a mask for one-half hour. There was no change in color of skin or mucous membranes. No change subjectively. Venous saturation before oxygen 67.7 per cent, after, 69.1 per

32 Barcroft, J. Anoxemia. *Lancet* 1: 487, 1920.

cent, venous carbon dioxide content before, 51.3 volume per cent, after, 53 volume per cent Pulse 66 before, 60 after Respiratory rate 14 before, 16 after Blood pressure 140/80 before, 135/85 after Vital capacity 3,564 c.c. before, 3,424 c.c. after Electrocardiogram showed normal rhythm before, and no change during or after oxygen

CASE 2 (Table 1)—Normal White male, physician, age, 26 years

EXPERIMENT—Oct 6, 1920 Subject in recumbent position, forty-five minutes after lunch Pure oxygen administered through a mask for one-half hour Arterial saturation before, 96.6 per cent, after, 99.6 per cent, venous saturation 71.3 per cent before Venous blood at site of administration was taken after wrist had been bandaged tightly at site of artery puncture, and hence blood was under stasis when drawn The saturation even under these conditions was 72.8 per cent

EXPERIMENT 2—Dec 15, 1920 Subject in recumbent position, four and one-half hours after breakfast Pure oxygen given through mask for one-half hour Pulse before, 76, after, 68 Respiration before, 19, after, 21 There seemed to be deeper breathing during oxygen Venous oxygen saturation 73.5 per cent before, 83 per cent after Venous carbon dioxide content 52.4 volume per cent before, 50.2 volume per cent after

In a normal man, the inhalation of oxygen for one-half hour increased definitely the oxygen content and saturation of arterial and venous blood In a second individual, the inhalation of oxygen for the same period increased the oxygen content and saturation of venous blood to a very slight extent The carbon dioxide content of venous blood was not markedly or consistently altered, although slight changes occurred in each experiment These may have been produced by a change in breathing incident to the mask Subjectively, there were no effects Objectively, the slowing of the pulse was the only other definite finding The blood pressure, vital capacity, rate of respiration, and electrocardiogram showed no significant changes

REPORT OF CASES

CASE 3 (Table 2)—T. M., white, male, coachman, age, 57 years

Diagnosis—Cardiac insufficiency, chronic myocarditis, partial heart block, right bundle branch block

History—Rheumatic fever when young, without cardiac symptoms Ten years alcoholic history Fifteen months before this admission, patient first noticed dyspnea and swelling of the legs Since then he has never been well, and for the six weeks before entry he has suffered from dyspnea, orthopnea and swelling of the legs and abdomen

Physical Examination—Oct 15, 1920 Orthopnea and periodic respiration Slight cyanosis of lips and mucous membranes Moderate distention of neck veins Heart much enlarged, apex in sixth space in anterior axillary line Sounds regular, rapid, of poor quality Systolic murmur over entire precordium Pulse full, rate 90 Artery wall thickened Blood pressure, 158/105 Dulness, diminished breath sounds, and moist râles at both bases Shifting dullness in abdomen Marked edema of legs Irrational most of the time Temperature normal Urine Specific gravity, from 1.022 to 1.030, slight trace of albumin Blood Wassermann negative Enlarged heart with widened arch on roentgenogram Electrocardiogram showed delayed conduction and right bundle branch block Nonprotein nitrogen 45.6 mg per 100 c.c.

Course and Treatment—Confined to bed, fluids restricted to 1,500 cc, digipuratum, 6 grains daily from Nov 15 to 20, 1920

On the night of admission, 400 cc of blood was removed, with a little relief

Oct 16, 1920 Condition same as on admission At 10 a m, oxygen saturation of the arterial blood was 87.5 per cent,³³ the venous saturation 53.2 per cent At 2 30 p m, the venous saturation was 41.6 per cent The only change was that he was more irrational in the afternoon Pure oxygen was given through a mask uninterruptedly for one-half hour At the end, the arterial saturation was 99.5 per cent, the venous saturation, 68.4 per cent Fifteen minutes after the administration, the venous saturation was 67.4 per cent The patient took the mask without complaint, and said he felt more comfortable after the inhalation Periodic respiration was present before oxygen After four minutes breathing became regular, of moderate depth, rate 30 per minute One minute after removal of mask periodic respiration recurred Cyanosis of lips disappeared and the ears became pink during the inhalation Pulse 90 before, 84 immediately after, 84 fifteen minutes after oxygen

October 17 No cyanosis evident Periodic respiration Pulse 60

October 19 Slight cyanosis of lips Periodic respiration Pulse 64

October 20 Face a grayish dusky color, lips slightly cyanotic Periodic respiration with long periods of apnea Distention of neck veins, congestion in lungs, and edema of extremities were undiminished Pulse from 40 to 50 Digitalis was stopped, 30 grains of digipuratum having been administered since admission Oxygen was given through a mask for one-half hour Arterial saturation before oxygen 83.5 per cent, one and one-half hours after, 89.9 per cent Venous saturation before, 53.7 per cent, at the end of fifteen minutes oxygen, 71.6 per cent, at the end of thirty minutes oxygen, 72.4 per cent, one and one-half hours after the inhalation, 72.4 per cent During the administration, his face noticeably pinkened, and dilated capillaries and venules stood out a bright red After one minute's oxygen, the periodic respiration was changed to regular breathing, rate 32, finally slowing to 25 Five minutes after the administration, it was still regular, rate 26 Fifteen minutes later, Cheyne-Stokes respiration had recurred, with, however, short periods of apnea, in contrast to the long apneic periods present before oxygen The pulse exhibited striking variations At the start it was regular, rate 48 (digitalis effect) During the inhalation it became very irregular, rate rising to 88 until the end of the administration, when it again became regular, rate 68 Five minutes later, it was 48 and regular

October 21 Patient was much quieter, more comfortable and slept better Cheyne-Stokes respiration continued most of the time with short periods of apnea, rate 26 No cyanosis Dilated venules and capillaries on face remained pink Pulse from 50 to 60 Venous saturation 78.1 per cent Venous carbon dioxide content 61.4 volume per cent

October 23 No cyanosis, but face was pale Signs of congestion in lungs less marked Edema of legs a little diminished

October 25 General condition unchanged Cheyne-Stokes breathing present Arterial saturation 96.6 per cent, venous saturation 77.4 per cent Arterial carbon dioxide content, 56.2 volume per cent, venous carbon dioxide content, 61 volume per cent Oxygen was given for one-half hour, a valve effect in the mask being utilized to provide about 70 or 80 per cent mixture The mask was first connected with the room air In one minute, periodic respiration was replaced by regular breathing, rate 33 Air was breathed for six minutes, and respiration continued regular, rate from 33 to 36 per minute

³³ The attempt was made to draw the arterial blood equally during apnea and dyspnea, but this could only be crudely approximated That the phases of periodic respiration make an important difference in oxygen content is shown by subsequent determinations

Oxygen was then given, and the breathing continued at about the same rate. His color previous to oxygen was pale, without cyanosis. After oxygen, his nose, cheeks, lips and ears were pink, and the dilated capillaries and venules became prominent and red. The pulse again showed interesting variations. Prior to the giving of oxygen, the rate varied with the phase of respiration, in dyspnea 48 per minute, in apnea 84 per minute (Duration of apnea fifteen seconds). During the inhalation the pulse rate quickened, and became very irregular, the irregularity being felt at the wrist as dropped beats, rate 88. Five minutes after oxygen, the pulse rate was 73, without dropped beats but varying slightly with the Cheyne-Stokes respiration, which had returned at this time. Shorter periods of apnea, however, were present, and the pulse variations were likewise smaller than before oxygen. At the end of the administration, the patient was asked to lie flat to see whether orthopnea persisted in the presence of a plentiful oxygen supply. He complained of immediate discomfort, and was allowed up. Patient was not clear mentally, and this response may have been a habit sensation rather than actual discomfort. The arterial saturation, taken forty minutes after the treatment, was 97.7 per cent, the arterial carbon dioxide content 59.2 volume per cent. The vital capacity immediately before oxygen was 1,194 c.c., immediately after, 1,166 c.c., two hours later, 1,166 c.c.

October 29 Clinical condition little changed. For past four days he has been allowed up in chair part of the day in attempt to restore clear mentality. Vital capacity 1,530 c.c.

October 31 Got up at night, walked about, fought efforts to replace him.

November 1 Temperature 101.5 F. Patient developed an acute bronchitis. Face had a dull grayish appearance, there was moderate cyanosis of lips and chin, and slight cyanosis of ears. Frequent productive cough present. He was more irrational. Cheyne-Stokes respiration continued. Pulse 100 in apnea, 80 in dyspnea. Vital capacity 942 c.c. Lungs showed scattered sibilant and sonorous râles over both chests, with dulness, diminished breathing and moist râles at right base. Mask was connected to room air to test effect on breathing. No change occurred, Cheyne-Stokes breathing persisting for entire fifteen minutes of experiment. An attempt was made to get arterial blood separately in apnea and dyspnea but the syringe filled too slowly to get unmixed samples. The arterial saturation obtained in apnea was 85.2 per cent, in dyspnea 87 per cent, the arterial carbon dioxide content in apnea was 56.7 volume per cent, in dyspnea 59.3 volume per cent.

November 3 Temperature fell to 99.5 F. Periodic respiration present with periods of apnea lasting from 15 to 20 seconds. Samples of arterial blood were obtained with less overlapping today. The arterial saturation in apnea was 78.3 per cent, in dyspnea 85.2 per cent. The arterial carbon dioxide content in apnea was 61.4 volume per cent, in dyspnea 62 volume per cent. Oxygen was given through a mask for one-half hour. At the start, the mask was connected with the room air, and in one minute the respiration became regular. When the mask was removed, Cheyne-Stokes breathing immediately recurred. The mask was then adjusted connected with oxygen, and in one minute the breathing again became regular, rate 34. At the end of the administration the cyanosis had completely disappeared. The capillaries and dilated venules on the face stood out bright red. The venous saturation was 94.5 per cent. The arterial saturation was not tested, but was obviously raised to the normal or above. Before oxygen the pulse was 96 in apnea and 56 in dyspnea. The electrocardiogram showed auriculoventricular block and right bundle branch block, in the dyspneic phase, sino-auricular slowing and increase of auriculoventricular block, in the apneic phase, increase of sino-auricular rate without dropped beats. During oxygen administration, with regular breathing, the pulse was regular, rate 84 to 92. The electrocardiogram confirmed the regularity, showed an absence of the marked auriculoventricular block, a decrease in the height of the R wave and less notching of the R complex.

Ten minutes after the administration, periodic respiration began again, though with much shorter periods of apnea. Pulse 88 in apnea, 74 in dyspnea. The electrocardiogram preserved the characteristics it had during oxygen administration. One-half hour later, pulse and electrocardiographic findings were the same. Two hours later, pulse and respiratory variations were the same as after oxygen. Immediately after oxygen, patient noticed no subjective relief, two hours later he said he breathed more easily. No cyanosis was evident. Blood pressure before and after inhalation 150/110. Vital capacity before, 942 cc, after, 858 cc.

November 4. No cyanosis evident. On the following morning he was removed to a chronic cardiac convalescent hospital. He died Nov 8, 1920.

In this case, a diminished oxygen saturation was present in both arterial and venous blood. The arterial (anoxic) anoxemia may be accounted for by the edema and passive congestion at the bases of the lungs, the venous (stagnant) anoxemia by the insufficient circulation. Oxygen inhalation raised the saturation of the arterial and venous blood to the normal level. The elevation of the arterial oxygen content naturally causes a corresponding elevation of the venous content, the respiratory exchange taking place at a higher level. In addition to this relative increase of venous oxygen, there was an additional increase that seems best explainable on the basis of an improved blood flow. The relative permanency of the elevated venous saturation tested at frequent intervals over a period of several days is further evidence that the circulation was improved by the increased supply of oxygen. The objective changes were in a measure confirmatory. The cyanosis disappeared for several days at a time. The pulse when regular was slowed. When the heart was under the influence of digitalis or of the phases of periodic respiration, the changes were more complicated. The periodic respiration was temporarily changed to regular respiration by the inhalation of oxygen. However, in two out of three trials, the mere application of the mask connected with room air was sufficient to cause regular respiration. No significant change in blood pressure, vital capacity, urinary secretion, or carbon dioxide content of arterial and venous blood resulted from oxygen inhalation in the amount given. The electrocardiographic changes indicate a lesser degree of block during and following the administration of oxygen. Subjectively, he usually said that his breathing was better or that he felt more comfortable, but he was never enthusiastic. It seemed that the mask contributed somewhat to lessening his sense of relief, as it was often hot and wet with perspiration at the end of the administration.

CASE 4 (Table 2)—G. W., colored, male, fireman, age, 57 years.

Diagnosis—Cardiac insufficiency, syphilitic aortitis, aortic regurgitation, right hydrothorax.

History—Rheumatism at 7, chancre at 23. In good health up to four years ago, when dyspnea, palpitation of heart and cough first occurred. During the months before admission he suffered from increasing dyspnea and orthopnea, massive edema of extremities and progressive distention of abdomen.

Physical Examination—Oct 4, 1920 Periodic respiration alternated with frank dyspnea No cyanosis of mucous membranes Heart much enlarged, downward and to the left Blowing diastolic murmur over aortic area, and systolic murmur at apex, rate, 88 Corrigan and capillary pulse Blood pressure, 190/60 Dulness and moist râles at bases of lungs, with diminution of breath sounds on right Massive edema of extremities Abdomen distended, no free fluid Liver not felt Urine showed a trace of albumin, specific gravity from 1.019 to 1.030 Phenolsulphonephthalein excretion 45 per cent in two hours Nonprotein nitrogen, 30 mg per 100 c c Blood Wassermann strongly positive

Course and Treatment—Confined to bed, restricted fluids, digipuratum from October 6 to 8, in all 8 grains, evening sedative of morphin or veronal

October 7 Patient in hospital three days with no change in physical signs or any improvement in comfort Oxygen was given through a mask attached to rebreathing apparatus for one-half hour Oxygen saturation of arterial blood before administration, 87.6 per cent, after, 99 per cent Venous saturation before, 71 per cent, after, 78.5 per cent Pulse 84 before, 82 after Respiration 34 before, 40 after Objectively there were no other changes Immediately after administration, he said he felt about the same In the evening he felt distinctly better and thought the oxygen had helped him Sleep was better that night than previously

October 8 Decided diuresis occurred Urinary output increased from 360 to 960 c c, with an intake less than usual, 480 c c, instead of the average daily intake of 800 c c Digipuratum had been given on three previous days, 8 grains in all

October 10 Patient slightly irrational Marked bradycardia and vomiting resulted from digitalis Pulse, though regular, had been brought from 88 to 48 He was very uncomfortable Oxygen was given through mask for one-half hour Venous saturation before, 57.3 per cent, at the end of fifteen minutes, 86 per cent, at the end of thirty minutes, 91.4 per cent Periodic respiration was replaced after one minute's oxygen by regular rapid respiration, rate 40 per minute Pulse remained low, between 45 and 50 Subjective response was that he felt a little sleepy at the end of the administration No diuresis followed

October 22 His course has been apparently stationary He showed at this time most of the evidences of cardiac insufficiency that he did on admission, including persistence of lung signs The oxygen saturation of the arterial blood was 89.9 per cent, the venous saturation 69.4 per cent He was removed to his home on the following day He died there one month later

In this case of decompensated syphilitic heart disease, the arterial (anoxic) anoxemia was fully corrected by the inhalation of oxygen The venous saturation, within normal limits after three days' rest in bed, was increased by oxygen inhalation The marked slowing of the heart due to digitalis was attended with a fall in venous saturation, apparently to the degree of a stagnant anoxemia, and suggests that the use of digitalis in regular hearts to the point of marked slowing causes a diminution in circulatory efficiency

CASE 5 (Table 2)—M T, white, male, tailor, age, 68 years

Diagnosis—Cardiac insufficiency, chronic myocarditis, auricular fibrillation, auriculoventricular block, right bundle branch block, emphysema, chronic bronchitis, polyserositis

History—No rheumatic or venereal history For many years previous to 1911 he took two glasses of brandy daily During the past thirteen years he

has suffered from cough and dyspnea, which have gradually but progressively become worse, until now he is practically bedridden. Cyanosis has been evident for five years. His abdomen has been tapped repeatedly for four years, at first every three months, now every three weeks. He lives at home, coming to the hospital only for paracentesis.

Physical Examination—Eight years ago he had an enlarged, regular heart without evidence of valvular disease, enlarged liver and spleen, emphysema and chronic bronchitis. His heart is now of huge size, extending into the mid-axilla. Electrocardiographic tracings have shown progressive changes, first a lengthening of the P-R interval, then right bundle branch block (slight alteration in the arteriogram, and finally auricular fibrillation). The other conditions also advanced, but to a less degree. The cyanosis was the most striking feature of the case, and he was called "the blue man."

Nov 9, 1920. At rest on a couch, he was moderately dyspneic. Ears, cheeks and nose were a reddish blue, lips a dark, deep blue, hands a bluish bronze color. The tips and borders of his tongue were purple. He was able to lie flat for two and one-half minutes, after which he became very uncomfortable. Lying flat was attended with an even greater degree of cyanosis. Oxygen was given through a mask for two five-minute periods, but he complained that it suffocated him. The rubber mouthpiece was then tried (for the first time) and he breathed through it readily. This was given for twenty minutes, the nostrils held occluded most of the time. The ears and nail-beds of the fingers pinkened slightly. The cheeks and hands were still markedly cyanotic, though less so than previously. The lips were still darkly cyanotic. The venous oxygen saturation before the administration was 29.6 per cent, twenty minutes after it was 82.4 per cent. Venous carbon dioxide content was 58.4 volume per cent before, 47.4 volume per cent twenty minutes after. Pulse (no deficit) from 50 to 64 before, from 54 to 66 after. Respiration, from 24 to 26 before, 28 after. Blood pressure, 115/55 before, 112/56, after. Vital capacity of the lungs 1,026 cc and 1,222 cc before, 1,110 cc after. Urine acid before, alkaline one-half hour later. Electrocardiogram showed auricular fibrillation and right bundle branch block. After oxygen, the notching of the R wave was less marked, and the height of the R wave was lowered. After the inhalation the patient (without any questioning) expressed decided increase in his general comfort and in his breathing.

November 18. Patient was seen in his home. He had an acute exacerbation of his chronic bronchitis. Respiration was accompanied by much wheezing. Sibilant and sonorous râles heard throughout both chests. Cyanosis very marked. Abdomen was more distended with fluid. Oxygen was given through the mouthpiece. A Douglas bag had been previously filled with a 60 per cent oxygen mixture, and left at the bedside in place of an oxygen tank. A two-way valve was inserted in the circuit, and patient gave oxygen to himself, twice a day for forty-five minutes at a time. This was repeated on the following day.

November 20. Cyanosis noticeably diminished by oxygen of two previous days. Dyspnea was still present, and abdomen was accumulating more fluid. Oxygen was again given as before, except that a 75 per cent oxygen mixture was supplied. Breathing was again described by the patient as "easier" after oxygen. Cyanosis was strikingly diminished in hands and ears, and finger nails were slightly pink. Lips were still blue, though less so than previously. Arterial oxygen saturation before today's inhalation was 86.3 per cent, one hour after, 95.6 per cent. Venous saturation, 46.4 per cent before, two days later, 57.8 per cent. The arterial carbon dioxide content was 47.7 volume per cent before, 49.9 volume per cent one hour later. The venous carbon dioxide content was 53.2 volume per cent before, and 53 volume per cent two days later. Pulse 60 before treatment, 48 one hour later, respiration 22 before, 23 one hour later.

November 22 No oxygen given for two days Acute bronchitis largely dissipated Color like that one hour after oxygen two days ago Abdomen distended with fluid and respiration was difficult Arterial saturation 97.8 per cent, venous saturation 57.8 per cent Arterial carbon dioxide content 52.6 volume per cent, venous carbon dioxide content, 53 volume per cent Pulse 50, respiration 24

November 24 Patient seen after paracentesis Felt much relieved Breathing better after removal of fluid Fingers and hands showed only slight cyanosis He said his hands had not been so free from cyanosis for years and reiterated that the oxygen treatment at his home made him much more comfortable and improved his breathing

Patient was not treated further with oxygen He continues to come to outpatient clinic for paracentesis

This case is an example of chronic arterial (anoxic) and venous (stagnant) anoxemia, the former element being due to emphysema, chronic bronchitis, and passive congestion at the bases of the lungs, and the latter to a slowed, insufficient blood flow It is a striking illustration of the fact that a severe lack of oxygen, even of anoxic type, may last for years without disastrous effects, if it supervenes very gradually The saturation of the arterial and venous blood was raised to the normal by the administration of oxygen The venous saturation in auricular fibrillation changes from minute to minute depending on the output from the heart, but the change in these experiments was too great to be accounted for in this way The difference in color of the blood before and after oxygen, from an almost black to a bright red, was more striking in this case than in any of the others studied A certain amount of permanency in the raised oxygen content of the blood and in the clinical improvement occurred The electrocardiographic changes again involving intraventricular block may, perhaps, indicate a lesser degree of block during oxygen administration It is interesting that the cyanosis was only little altered after the first single administration of oxygen, but became definitely relieved after continued administration, although in each case the blood values showed great improvement

CASE 6 (Table 2)—L. J., white, male, cabinet maker, age, 62 years

Diagnosis—Myocardial insufficiency, auricular fibrillation, cardiac hypertrophy

History—No rheumatic, tonsillar, or venereal history Ten years ago, first suffered from dyspnea on exertion, palpitation and precordial pain Never had edema of extremities Three subsequent attacks occurred Present break in compensation is similar to previous ones

Physical Examination—March 21, 1921 Face deeply flushed Lips red, with cyanotic overcast Finger nails, cheeks and ears slightly cyanotic No dyspnea at rest Heart markedly enlarged, completely irregular, without murmurs Blood pressure, 170/100 Moist râles at both bases, diminished breath sounds at right base, and bronchovesicular breathing at left back below angle of scapula Liver edge palpable 5 cm below costal margin Urine specific gravity, from 1.014 to 1.020, very slight trace of albumin Blood

Wassermann negative Nonprotein nitrogen, 41.4 mg per 100 cc Phenol-sulphonaphthalein excretion, 35 per cent in two hours Red blood cell count, 6,100,900 Hemoglobin, 154 per cent (oxygen capacity) Vital capacity, 1,754 cc

Course and Treatment—Confined to bed, salt-free low protein diet, convallaria, 5 cc, three times a day, March 25 and 26 (used in a separate investigation)

March 25, 1921 Condition unchanged Vital capacity 1,866 cc Oxygen given through mouthpiece for one-half hour Oxygen saturation of the arterial blood before the inhalation, 86 per cent, after, 98.1 per cent, venous saturation before, 53.4 per cent, after, 47.8 per cent Arterial carbon dioxide content before, 40.3 volume per cent, after, 41.3 volume per cent, venous carbon dioxide content before, 48.2 volume per cent, after 49.9 volume per cent The cyanosis gradually cleared, leaving the lips deep red, and giving an increased pinkness to rest of face No change of a consistent nature occurred in cardiac, pulse, or respiratory rate Electrocardiographic tracings showed auricular fibrillation unchanged, before and after The patient said he felt better after the inhalation He breathed about 80 per cent oxygen The cyanosis began to return directly after the administration, and in half an hour was again marked, not seemingly to the degree before oxygen On the following day, no effect of the oxygen inhalation could be seen clinically Vital capacity, 1,474 cc

March 29 Condition unchanged No dyspnea at rest Cyanosis was not definitely altered Arterial oxygen saturation 88.3 per cent, venous saturation, 58 per cent Arterial carbon dioxide content, 42.7 volume per cent Oxygen was given through a mouthpiece for one hour, patient breathing about 50 per cent oxygen Clinical effects were similar to previous administration Blood not tested Vital capacity before oxygen, 1,726 cc, day after oxygen, 1,838 cc

Patient left the hospital two weeks later Seemed to have less dyspnea on exertion Cyanosis was still present, a little less marked Signs in lungs largely persisted

The exact diagnosis of this patient remained in doubt The signs in the lungs were those of passive congestion, and apparent compression of the left lower lobe from the large heart The anoxemia was both arterial (anoxic) and venous (stagnant), and of marked degree in proportion to the other symptoms The chronic nature of the diminished oxygen content of the blood was manifested by the polycythemia Inhalation of oxygen completely relieved the arterial anoxemia, though only temporarily The lowered venous content of oxygen can be explained by the varying output from the heart occurring in auricular fibrillation Lundsgaard⁵ showed that the venous oxygen content in auricular fibrillation may be normal in the stage of decompensation, and also that it might be abnormally lowered in full compensation It is obvious that the venous saturation cannot be used as a criterion of oxygen therapy when the auricles are fibrillating

CASE 7 (Table 2)—S M, white, housewife, age, 68 years

Diagnosis (necropsy)—Cardiac insufficiency, cardiac hypertrophy and dilatation, pulmonary edema, pulmonary thrombus

History—Shortness of breath and fatigue for two years Two months before entry, patient began to suffer from a sense of suffocation, and pronounced dyspnea and orthopnea Swelling of legs and ankles, continued nausea, and general weakness caused her to enter the hospital

Physical Examination—Oct 25, 1920 Dyspneic and orthopneic Slight cyanosis of mucous membranes Lungs, anteriorly and posteriorly, filled with fine and medium moist râles Heart much enlarged, downward and to left Sounds muffled, regular, without murmurs Blood pressure, 190/110 Pulse full and bounding, rate 78, no thickening of radial artery Veins of neck extremely distended Fluid wave and shifting dullness in abdomen Massive leg edema Phenolsulphonephthalein excretion 30 per cent in two hours Non-protein nitrogen 40 mg per 100 cc of blood Urine, specific gravity, from 1.020 to 1.022, trace of albumin, occasional cast White blood cells, 17,000, polymorphonuclears, 90 per cent Blood Wassermann, negative

Course and Treatment—Confined to bed, fluids restricted to 800 cc, digipuratum, October 25 to 28, in all 20 grains

Oct 27, 1920 Condition unchanged Patient was very restless and anxious Arterial saturation, 90.7 per cent, venous saturation, 69 per cent Arterial carbon dioxide content, 39 volume per cent, venous carbon dioxide content, 45.2 volume per cent

October 28 Dyspnea continued Face was of a grayish dusky color, with moderate cyanosis of the lips Râles in lungs were of coarse moist variety Arterial saturation, 88 per cent, venous saturation, 74.5 per cent Arterial carbon dioxide content, 43.5 volume per cent, venous carbon dioxide content, 46.5 volume per cent Oxygen was given through a mask connected with a Douglas bag containing a 60 per cent oxygen mixture Three fifteen-minute periods were given, separated by five-minute intervals Patient took the mask poorly, and it was stopped because of the discomfort it gave her One-half hour later, the arterial saturation was 90.9 per cent, the arterial carbon dioxide content, 43 volume per cent No improvement in color, respiration, pulse or distention of neck veins was observed On questioning, she said she breathed easier after the oxygen She was then given pure oxygen for three five-minute periods, after which the administration was finally stopped because of patient's discomfort in breathing through a mask Pulse 90 before oxygen, 104 after Respiration 27 before, 36 after Vital capacity, 672 cc, before, 450 cc after Blood pressure, 205/118 before, 210/120 after There seemed to be the very slightest alleviation of the cyanosis of the lips as a result of the oxygen No further administration was attempted She became increasingly cyanotic, and died at five the next morning

At necropsy a large hypertrophied and dilated heart was found, without evidence of valvular disease There was sclerosis of the aorta, pulmonary and renal arteries In branches of the right pulmonary artery leading to the inferior lobe of the right lung, grayish-red thrombus masses were seen, with corresponding areas of lung infarction The lung tissue of both lungs was moist, leathery, and of a salmon pink color The trachea and bronchi were bathed in muco-pus

The anoxemia in this case was of the anoxic type, arising obviously from the congestion and edema of the lungs This was not successfully overcome by oxygen in the period given, although it was partially relieved The mask was so disturbing to the patient as to render its use impossible, and accounts for the elevation in pulse and respiration (The mouthpiece had not been thought of at the time this patient was studied)

CASE 8 (Table 2)—J V, white, housewife, age, 50 years

Diagnosis—Cardiac insufficiency, rheumatic mitral valvular disease, chronic myocarditis, auricular fibrillation, pulmonary edema (At necropsy, deformity of the mitral, tricuspid and aortic valves elevated fibrous plaque at beginning of right coronary artery were found)

History—Tonsillitis, followed by rheumatism, when 23 years old. For past sixteen years patient has had dyspnea on exertion. Nine days ago, was abruptly seized with palpitation of heart and dyspnea, followed the day after by substernal pain, vomiting, sleeplessness and weakness.

Physical Examination—Oct 19, 1920. Marked dyspnea and orthopnea. No cyanosis. Heart greatly enlarged, completely irregular, with systolic and diastolic murmurs at apex and at aortic area. Apex rate, 140, pulse rate, 128. Blood pressure, 120/85. Lungs clear. Liver not palpable. Slight edema over tibia. Urine specific gravity, from 1.026 to 1.028, slight trace of albumin. Blood Wassermann, negative. Phenolsulphonephthalein excretion, 20 per cent in two hours. Nonprotein nitrogen 60 mg per 100 cc. Electrocardiogram showed auricular fibrillation, left ventricular preponderance.

Course and Treatment—Confined to bed, soft solids, digifolin 4 cc, and digipuratum 9 grams, from Oct 19 to 26, 1920, evening, sedatives of morphin.

Oct 26, 1921. Course in hospital showed no improvement. On the previous night, restlessness and insomnia being unrelieved, hyoscin hydrobromid, 0.01 gram, was given. In the morning she was in coma, and outspoken edema of the lungs was present. Frothy mucus filled the mouth and throat. Face was a dusky gray, lips, hands and finger nails were deeply cyanotic. Respiration was moderately deep, rate 37. Pulse was scarcely palpable, completely irregular, and very rapid. Veins were collapsed. Oxygen saturation of the arterial blood was 76.1 per cent. Arterial carbon dioxide, 39.2 volume per cent. Because of agonal condition of patient, oxygen administration was not delayed to get venous saturation. Pure oxygen was given through a mask for two hours and twenty minutes. At the end of forty minutes the venous saturation was 43.8 per cent, taken while she was breathing oxygen. The venous carbon dioxide content was 52.2 volume per cent. No change clinically could be observed. At the end of one hour, the pulse became temporarily palpable, but quickly tapered off into a thready run which could not be counted. At the end of two hours, it became palpable for several minutes. At this time the lips showed a distinct diminution of cyanosis, and there was less cyanosis in the fingers and hands. No change in breathing was observed, and frothy mucus continued to collect in the back of the throat. Arterial oxygen saturation was 97.7 per cent, the arterial carbon dioxide content, 45.7 volume percent. At the end of two hours and twenty minutes the venous saturation was 59 per cent, the venous carbon dioxide content 55.2 volume per cent. The administration was then stopped. Face, lips, hands, finger nails, and upper portion of chest speedily became a deep blue, and she died twenty minutes later.

In this case, a severe arterial (anoxic) anoxemia was caused by widespread edema of the lungs, the venous (stagnant) anoxemia was caused by acute heart failure. Cyanosis showed no distinct relief until oxygen had been given for two hours. Arterial saturation at that time showed that the oxygen content of the arterial blood had been raised to the normal. The long time that was required to alleviate the cyanosis was a surprising feature. The abundance of foam and mucus in the bronchial tree and the existence of a venous anoxemia are probably accountable for this, since the former delayed the passage of the oxygen and the latter masked the improvement. The elevation of the venous saturation is probably largely explained by the raising of the arterial saturation. The carbon dioxide content of arterial and venous

blood steadily rose, indicating that the edema fluid hindered the respiratory exchange in the lungs. An acidosis was probably present due to carbon dioxide accumulation.

CASE 9 (Table 2)—H. R., colored, male, janitor, age, 45

Diagnosis—Cardiac insufficiency, chronic myocarditis, asthma, chronic bronchitis, pulmonary edema

History—No rheumatic or syphilitic history. One pint of whisky daily for many years. Shortness of breath and cough began three years ago. During the past six weeks cough, dyspnea and discomfort have been extreme, and extremities became swollen.

Physical Examination—Feb 3, 1921. Marked dyspnea, respirations rapid and shallow, rate 45, moderate cyanosis of mucous membranes. Lungs full of sibilant, sonorous and medium-sized moist râles, more in front than back. Heart much enlarged. Sounds regular, rapid, poor quality. No murmurs. Blood pressure, 145/105. Vessel wall palpable. Liver dullness extends to umbilicus in right midclavicular line. Moderate leg edema. Urine specific gravity, from 1.012 to 1.020, albumin, slight trace. Phenolsulphonephthalein excretion, 50 per cent in two hours. Nonprotein nitrogen, 74.1 mg per 100 cc. Blood Wassermann, negative.

Course and Treatment—Confined to bed, fluids restricted to 1,500 cc, digitalis leaves, 2 grains, three times a day, evening sedatives. Patient's course was stationary for seven days, and then took a sudden change for the worse.

Feb 10, 1921. He was found comatose in the early morning. Respirations rapid and shallow, rate 45. Lips and mucous membranes quite cyanotic. Pupils pin-point, and react very slightly to light. Pulse 128, small, easily compressible. Heart sounds not heard because of gurgling noises during respiration. Lower half of anterior chest is filled with medium and coarse moist râles, posteriorly moist râles are scattered throughout.

Oxygen was administered through the Haldane mask at the rate of four liters per minute for fifteen minutes. No change in condition was observed, and rebreathing apparatus was substituted, with valve attachment to the mask affording about 80 per cent oxygen. One-half hour later patient was bled 500 cc. Oxygen was kept up for three hours. Pulse had gradually fallen to 100, respirations varied between 36 and 44, cyanosis had disappeared. Patient at times moved about in bed, opened his eyes, and showed signs of returning consciousness. After oxygen had been administered for forty-five minutes the arterial blood showed a saturation of 100 per cent, the venous saturation was 53.6 per cent. Arterial carbon dioxide content was 75.0 volume per cent, venous carbon dioxide content, 82.4 volume per cent. The pulse rate remained down the rest of the day, and there was no cyanosis.

February 11. Patient looked much better in the morning. He was still dyspneic, but cyanosis was absent. He seemed rational and answered questions. Moisture in lungs continued. In the afternoon he swiftly became very cyanotic, dyspneic, and finally comatose. The oxygen saturation of his venous blood was 0.3 per cent, the carbon dioxide content, 63.4 volume per cent. Oxygen was administered as on the previous day. Cyanosis was much diminished, though still present. Pulse slowed from 112 to 100. Oxygen was stopped after two hours, and patient died one hour later.

Asthma, bronchitis, and edema of the bases of the lungs were responsible for the arterial (anoxic) anoxemia. The development of general pulmonary edema introduced a very grave state of oxygen want. The inhalation of oxygen plus venesection tided him over for one day. On the following day, a sudden recurrence of anoxemia,

largely due to cardiac failure and shown strikingly in the venous saturation, was only partially relieved by oxygen. Death occurred one hour after oxygen was stopped, due apparently to cardiac failure.

DISCUSSION

It is interesting to note that all of the patients had an arterial (anoxic) anoxemia, which we must attribute to the associated pulmonary conditions. Most of them had a stagnant type of anoxemia, which can be attributed directly to the insufficient output from the heart. The cases, it may be observed, were selected usually because of the presence of cyanosis and do not represent all varieties of cardiac insufficiency, since in some cyanosis is entirely absent.

These cases showed uniformly an increase in the oxygen saturation of the arterial blood following short periods of oxygen inhalation. When passive congestion and edema were localized at the bases, or in the presence of acute bronchitis or chronic bronchitis and emphysema, the relief of arterial (anoxic) anoxemia was comparatively prompt. When widespread edema of the lungs was present, more prolonged administration of oxygen was required to elevate the arterial saturation.

The mechanism of elevation of the arterial saturation seems to depend on the increased diffusion pressure of oxygen available in oxygen-rich mixtures. In the cases in which passive congestion and edema are confined to the bases, the arterial anoxemia arises presumably because the swelling and edema in the alveolar wall act as a hindrance to the normal diffusion of oxygen.

In widespread edema of the lungs, the fluid in the alveoli and bronchial tree inhibits the free passage of air. In acute bronchitis, excess moisture in the bronchi and bronchioles acts in a similar way. These hindrances to the normal exchange of oxygen are apparently overcome by an increased diffusion pressure of oxygen. A second factor to be considered in the elevation of the arterial saturation is the shallow character of the pulmonary ventilation and the related diminution of the vital capacity of the lungs that occurs in cardiac insufficiency. Shallow breathing, although brought about by anoxemia, is itself a cause of anoxemia.³⁴ That this plays any great rôle in the production of the anoxemia of heart disease or in its alleviation is at present in doubt. In this series, no significant changes in rate of respiration occurred except in the change from periodic to regular breathing. Beddard and Pembrey³⁵ found a decrease in pulmonary ventilation

³⁴ Haldane, J. S., Meakins, J. C., and Priestley, J. G. The Effects of Shallow Breathing, *J. Physiol.* **52** 449, 1919.

³⁵ Beddard, G. P., and Pembrey, M. S. Observations on Pulmonary Ventilation in Disease, *Brit. M. J.* **2** 580, 1908.

during oxygen inhalation in four cases of cardiac failure, without change in rate. If this is a constant occurrence, oxygen inhalation does nothing in the way of removing shallow breathing, but merely increases its effectiveness. According to Haldane, Meakins and Priestley³⁴ however, shallow breathing may arise in heart disease with impaired circulation as a direct result of the anoxemia. In their experiments on normal individuals it was found that shallow breathing caused uneven ventilation of the lungs, which in turn caused anoxemia, periodic respiration and other symptoms. These effects were made worse when the subject assumed the recumbent position, and this was made the basis of the explanation of orthopnea in cardiac insufficiency. They state that "since the orthopnea is due to shallow breathing caused by and intensifying anoxemia, it is clear that the condition can be treated by adding oxygen to the air inspired." In the cases of our series, it frequently seemed that the breathing was deeper during oxygen inhalation, but since no exact measurements were made no conclusions can be drawn, and it remains for further investigation to determine the precise rôle of shallow breathing in heart disease. It is our impression that it may augment a previously existing arterial anoxemia, but that it is not ordinarily of the extent to be the primary cause.

Periodic respiration has been considered a manifestation of slight degrees of oxygen want. In these cases, it was constantly changed to regular breathing by oxygen administration. However, immediately after the mask was removed, with the blood still normally saturated with oxygen, the periodic respiration usually recurred. Further, it was sometimes changed to regular respiration by the application of the mask connected with the room air. Although oxygen deficiency is probably its essential cause, there are other factors such as the patient's attention and habit, which operate in its continuance.

Cardiac dyspnea in the cases studied seemed to have little relation to the degree of anoxemia, either anoxic or stagnant. Further, the inhalation of oxygen, although it completely relieved the anoxemia, produced only slight or moderate relief of the dyspnea. In accordance with these facts, we hold the belief, expressed by previous investigators, that lack of oxygen is not the important cause of cardiac dyspnea, even in the presence of marked cyanosis. It seems rather to be regarded as an important and dangerous byproduct. The carbon dioxide contents of the arterial and venous blood in three cases showed a decrease in alkali reserve, and in two cases an apparent increase. As a result of oxygen therapy, there was frequently a slight increase in carbon dioxide content. In a recent study of the carbon dioxide dissocia-

tion curve in cardiac decompensation, Peters³⁶ expresses the belief that the cause of the dyspnea is the fact that a greater ventilation is necessary to effect the normal carbon dioxide elimination, with the additional factors in some cases of a carbon dioxide acidosis (with actual lowering of the p_H), and in others a reduction of the available alkali of the blood. In four tested cases, an increase in the normal difference between the arterial and alveolar carbon dioxide tension was found. That an inefficient pulmonary mechanism for the exchange of the blood carbon dioxide is at the basis of cardiac dyspnea is indeed strongly suggested by Peters' work. That a mechanical factor, the diminution of the vital capacity emphasized by Peabody,³⁷ is the important cause of the inefficient pulmonary ventilation involves, it seems to us, a necessary addition to the theory of cardiac dyspnea.

The venous saturation was regularly increased by oxygen inhalation except in one case of auricular fibrillation. In this condition, the varying output from the heart results in a variable venous saturation, which, therefore, becomes an unreliable criterion for oxygen therapy, just as it is of circulation efficiency. The elevation of the venous saturation in the main represented and was due to the elevation in arterial saturation, the respiratory exchange taking place on a higher level. In addition to this relative increase of venous oxygen, there was in three cases a further increase that seems best explained on the basis of an improved blood flow. In two cases tested at frequent intervals, the elevated venous saturation had a certain degree of permanency, which again suggested that the circulation was bettered by the increased supply of oxygen. The relief of the cyanosis and the slowing of the pulse were in a measure confirmatory.

It must be recognized that a venous saturation below 65 per cent does not indicate that the circulation is impaired, unless the arterial saturation is normal. In order to determine whether there is circulatory defect, the difference between the arterial and venous saturation must be obtained and compared with the normal difference. If it is increased, an inefficient circulation is indicated. This difference has been emphasized by Means and Newburgh⁴ as the significant determination in the estimation of blood flow from oxygen values, and was first discussed by Krogh and Lindhard³⁸ and termed by them the coefficient of utilization of the oxygen carrying power. The normal

36 Peters, J. P., and Barr, D. P. The Carbon Dioxide Dissociation Curve and the Carbon Dioxide Tension of the Blood in Cardiac Dyspnea, *J. Biol. Chem.* **45** 559, 1921.

37 Peabody, F. W., and Wentworth, J. A. The Vital Capacity of the Lungs and Its Relation to Dyspnea, *Arch. Int. Med.* **20** 443, 1917.

38 Krogh, A., and Lindhard, J. Measurements of the Blood Flow Through the Lungs of Man, *Skand. Arch. f. Physiol.* **27** 100, 1912.

range is between 20 and 30 per cent, since the normal arterial saturation is about 95 per cent and the venous saturation between 65 and 75 per cent. The determination of cardiac insufficiency on the basis of the oxygen unsaturation in volume per cent or the oxygen consumption in volume per cent, introduces a factor which has little relation to blood flow, namely, hemoglobin. The upper limit of normal for these values has been placed by Lundsgaard⁵ and Harrop⁸ at 8 volume per cent. Now, if a patient's hemoglobin registers 43 per cent according to the Haldane scale, his oxygen capacity is only 8 volume per cent. In order for him to show any cardiac insufficiency on this standard the oxygen unsaturation would have to be over 8 volume per cent, which is manifestly impossible as he does not possess

TABLE 1—EFFECT OF OXYGEN ON THE BLOOD GASES IN TWO NORMAL INDIVIDUALS *

Case No	Diagnosis	Date, 1920	Oxygen Inhalation	Oxygen Content		Oxygen Saturation		Oxygen Capacity Vol %	Hemoglobin Calculated from Oxygen Capacity	Carbon Dioxid Content	
				Arterial Vol %	Venous Vol %	Arterial %	Venous %			Arterial Vol %	Venous Vol %
1	Normal	11/18	Before oxygen		11.52		67.7	17.03	92.1		51.3
			Oxygen for 30 min		11.78		69.1	(17.03)	(92.1)		53.0
2	Normal	10/6	Before oxygen	16.79	12.38	96.6	71.3	17.38	94.0		
			Oxygen for 30 min	17.30	12.64	99.6	72.8†	(17.38)	94.0		
	Normal	12/15	Before oxygen		14.63		73.5	19.92	107.0		52.4
			Oxygen for 30 min		16.51		83.0	(19.92)	(107.0)		50.2

* Subject in recumbent position.

† At end of oxygen administration wrist was tightly bandaged at site of artery puncture, and hence venous blood in this instance was under stasis when drawn.

the available hemoglobin. For an estimation of blood flow, then, the venous unsaturation in volume per cent would be accurate only to individuals with a normal hemoglobin, whereas the venous saturation in per cent and the coefficient of utilization would hold good for all patients since it gives the relative usage of the hemoglobin.

The slowing of the pulse that occurred in the normal individuals occurred to a greater extent in the presence of anoxemia. Slowing of the heart in normal men was first observed by Smith³⁹ and recently carefully confirmed by Benedict and Higgins.²⁹ The blood pressure and vital capacity showed no immediate relation to oxygen deficiency or its correction. The electrocardiogram in the normal and in a case of uncomplicated auricular fibrillation showed no alteration. In two

³⁹ Smith, H. A. The Effect of Oxygen Inhalation on the Pulse, *Med Rec* 1:481, 1871.

TABLE 2—EFFECT OF OXYGEN INHALATION ON THE BLOOD GASES IN SEVEN CASES OF CARDIAC INSUFFICIENCY

Case No	Diagnosis	Date	Oxygen Inhalation*	Oxygen Content		Oxygen Saturation		Oxygen Capacity Vol %	Hemoglobin Calculated from Oxygen Capacity	Carbon Dioxide Content		Remarks
				Arterial Vol %	Venous Vol %	Arterial %	Venous %			Arterial Vol %	Venous Vol %	
3	Cardiac insufficiency, chronic myocarditis, partial heart block, right bundle branch block	10/16/20	4 hrs before oxygen	16.52	10.05	87.5	73.2	18.00	102.1			Periodic respiration
			Before oxygen	19.71	8.16	99.5	41.6	19.62	106.1			Regular respiration
		10/20/20	15 min after oxygen		13.41		68.4	(19.62)	(106.1)			
			Before oxygen	18.29	11.73	83.5	77.7	21.88	118.2			Periodic respiration
		10/21/20	Oxygen for 15 min		15.69		71.6	(18.8)	(118.2)			Regular respiration
			Oxygen for 30 min	19.68	15.81	89.9	72.4	(21.88)	(118.2)			Regular respiration
		10/23/20	1½ hrs after oxygen		15.91		78.1	(21.88)	(118.2)		61.1	Periodic respiration
			24 hrs after oxygen		12.55		63.1	20.10	110.1			Periodic respiration
		10/25/20	Before oxygen	18.27	14.62	96.6	77.1	19.00	102.1		61.0	Periodic respiration
			40 min after oxygen	17.94		97.7		18.39	99.4			Periodic respiration
4	Cardiac insufficiency, syphilitic aortitis, aortic regurgitation, right hydrothorax	11/ 1/20	No oxygen—apnea	15.44		85.2		18.12	98.0			Acute bronchitis developed
			dyspnea	17.78		87.0					65.1	Cyanosis +++
		11/ 3/20	No oxygen		9.73		53.6	(18.12)	(98.0)			Cyanosis +++
			Before oxygen—apnea	15.76		78.3		20.17	108.9		61.4	Cyanosis +++
		10/ 7/20	dyspnea	17.14		85.2		(20.17)	(108.9)		62.0	Cyanosis 0
			Oxygen for 30 min	15.70	19.08	87.6	91.5	(17.90)	(97.1)			After 3 days in bed
		10/10/20	Before oxygen	17.71	12.72	99.0	78.5	(17.90)	(97.1)			Excess of digitals
			Oxygen for 30 min		14.04		71.0	20.31	109.8			
		10/22/20	Before oxygen		11.62		73.3	(20.31)	(109.8)			
			Oxygen for 30 min	14.16	17.45	89.9	91.4	(20.31)	(109.8)			
5	Cardiac insufficiency, chronic myocarditis, auricular fibrillation, auriculoventricular block, right bundle branch block, emphysema, chronic bronchitis	11/ 9/20	No oxygen		10.91		69.4	15.77	55.2		58.4	Oxygen for one half hour
			Before oxygen		6.32		29.6	21.32	115.1		47.4	Oxygen given on 2 previous days
		11/20/20	20 min after oxygen	18.24	17.58	86.3	82.4	(21.32)	(115.1)			
			Before oxygen	18.64	9.83	95.6	46.4	21.18	114.2		47.7	
		11/22/20	1 hr after oxygen	18.64	10.90	97.8	57.8	19.50	103.2		49.9	
			48 hrs after oxygen	24.56	15.23	86.0	53.4	18.89	102.0		52.6	
		3/25/21	Before oxygen	27.99	13.60	98.1	47.8	28.54	154.3		38.0	
			5 min after oxygen	23.34	15.33	88.3	58.0	(28.54)	(154.3)		40.3	
		3/29/21	No oxygen		14.73		69.0	26.45	143.0		41.3	Oxygen for one-half hour
			Before oxygen	19.14	16.20	90.7	74.5	21.10	114.0		42.7	Patient bled 300 cc day previous
6	Cardiac insufficiency, pulmonary edema, pulmonary thrombus	10/27/20	No oxygen		10.91		69.4	15.77	55.2		58.4	
			Before oxygen		6.32		29.6	21.32	115.1		47.4	
		10/28/20	Oxygen for 45 min	19.70	17.58	90.9	82.4	(21.32)	(115.1)			
			Before oxygen	16.38	9.83	86.3	46.4	21.18	114.2		47.7	
		10/26/20	Oxygen for 40 min	20.14	12.14	97.7	43.8	21.30	116.1		52.2	
			Oxygen for 2 hrs	19.14	10.01	100.0†	53.6	(20.60)	(111.2)		55.2	
		2/10/21	Oxygen of 2 hrs					18.70	101.0		82.4	Cyanosis relieved by oxygen
			Oxygen for 45 min									
		2/11/21	Before oxygen		0.06		0.3	19.88	107.2		63.4	Cardiac failure

The term "before oxygen" refers only to the day of the blood determination. It indicates that the blood for analysis was drawn before any oxygen therapy of the day, and that oxygen was subsequently given on the same day. "Oxygen for — min" indicates that the blood was taken immediately or a short time after oxygen therapy. All other terms as well, such as "no oxygen," refer solely to the day of the determination. For the precise relation of oxygen inhalation to the blood determination, as well as for the complete duration of the therapy, the text must be consulted. The parenthesis () about the figure for the oxygen capacity indicates that the determination preceding was used.

* There was apparently in excess of oxygen in physical solution, 0.44 vol %

cases of right bundle branch block, one with and one without auricular fibrillation, a decreased notching and a diminished height of the R wave was present during the inhalation of oxygen

The question of the effect of oxygen on renal function is being additionally investigated. No definite action on the quantity of urine eliminated was discerned in the short periods in which oxygen was given

SUMMARY

1 In a normal man, the inhalation of oxygen for one-half hour caused an increase in the oxygen saturation of the arterial and venous blood. In a second normal individual, the inhalation of oxygen for the same period caused a very slight rise in the venous saturation, the arterial saturation not being tested. The pulse was slowed in both cases. No significant changes occurred in the blood pressure, vital capacity, electrocardiogram, venous carbon dioxide content, or rate of respiration.

2 In seven cases of cardiac insufficiency, an anoxic (arterial) anoxemia was present in all, a stagnant (venous) anoxemia in all except one.

3 Oxygen inhalation regularly increased the arterial saturation. Where the anoxic anoxemia seemed due to passive congestion and edema at the bases of the lungs, the arterial saturation was raised to the normal by inhalation of oxygen for one-half hour. In the cases complicated by widespread pulmonary edema, relief of arterial anoxemia was accomplished in from forty-five minutes to two hours.

4 Oxygen inhalation increased the venous saturation in all except one case of auricular fibrillation. The elevation of the venous saturation was largely due to the raising of the arterial saturation. In a few cases, there was an additional and somewhat permanent increase in the venous saturation that seems best explained on the basis of an improved blood flow resulting from the increased supply of oxygen.

5 The arterial anoxemia of acute and chronic bronchitis, and emphysema, occurring in cardiac insufficiency, was fully relieved by oxygen inhalation. The venous saturation was correspondingly elevated.

6 The relief of the cyanosis and the slowing of the pulse were the outstanding objective changes. The blood pressure, vital capacity, arterial and venous carbon dioxide content, urinary excretion, and rate of respiration showed no definite changes from short periods of oxygen inhalation. The electrocardiogram showed consistent changes in two cases of right bundle branch block, no change in one uncomplicated

case of auricular fibrillation. Subjectively, the patients usually said they felt more comfortable or that their breathing was better, but they were rarely enthusiastic.

We wish to express our thanks to Dr. James H. Means for his invaluable aid during the work reported in this and the following two papers, and for his kind assistance in the preparation of the manuscript. We are greatly indebted to Dr. Donald D. Van Slyke and Dr. William C. Stadie who showed us the combined method of gas analysis before it was published, and to Dr. Paul D. White for his aid in obtaining and analyzing the electrocardiograms.

STUDIES IN OXYGEN THERAPY

II IN PNEUMONIA AND ITS COMPLICATIONS*

ALVAN L. BARACH, M.D.

AND

MARGARET N. WOODWELL, A.B.

BOSTON

In the previous paper, a report was made of the effect of oxygen on two normal individuals and seven cases of cardiac insufficiency. This paper represents a continuance of the studies in oxygen therapy in ten cases of lobar pneumonia and two cases of bronchopneumonia. The theoretical indications for giving oxygen, the methods employed, and the recent pertinent literature have been outlined in the beginning of the first paper. The plan of investigation has been maintained, namely, the correlation of the clinical condition of the patient with the oxygen saturation of the arterial and venous blood before and after treatment.

REPORT OF CASES

The individual cases follow.

CASE 10[†] (Table 3)—M. W., white, male, driver, 36 years.

Diagnosis—Lobar pneumonia, chronic pulmonary tuberculosis, edema of the lungs.

History—Mother and sister died of tuberculosis. Patient has suffered from a chronic productive cough for five years. Three days before entry, tiredness, headache, and sharp pain in left chest began. Two days later, cough increased in intensity, sputum resembled prune juice, and weakness became marked.

Physical Examination—Jan. 29, 1921 (fourth day of disease). Temperature, 103.0 F., pulse, 138, respiration, 32. Very poor nutrition. Moderate dyspnea. Marked cyanosis of nail beds. Consonating râles over entire left chest. Dulness and diminished breath sounds at left base. Dulness and bronchovesicular breathing below right clavicle. Heart not enlarged. Sounds regular, rapid good quality, without murmurs. Artery wall palpable. Blood pressure, 115/80. Urine, negative. Blood Wassermann, negative. White blood cells, 37,400, polymorphonuclears, 90 per cent. Blood cultures (two), sterile. Sputum, pneumococcus subtype II. Sputum for tubercle bacilli positive.

Course and Treatment—Routine pneumonia treatment (force fluids, soft solids, evening sedatives, usually morphin). Digitalis leaves, 1 grain, three times a day from Feb. 1 to 15, 1921.

Jan. 31, 1921 (sixth day of disease). Temperature, 102.3 F., pulse, 130, respiration, 30. Dyspnea and cyanosis as on admission. Patient mentally alert.

* From the Medical Service of the Massachusetts General Hospital.

[†] This paper is No. 16 of a series of studies of the physiology and pathology of the blood from the Harvard Medical School and allied hospitals.

The expense of this work was in part defrayed by a grant from the Proctor Fund of Harvard University for the study of Chronic Diseases.

1. The cases in the three papers are numbered serially.

and rational Arterial oxygen saturation, 77.2 per cent, venous saturation, 50.2 per cent Arterial carbon dioxide content, 49.5 volume per cent, venous carbon dioxide content, 53.1 volume per cent Carbon dioxide dissociation curve showed a slight acidosis²

February 1 (seventh day of disease) Temperature, 101.0 F, pulse, from 116 to 120, respiration, 32 Patient appeared distinctly worse Increased dyspnea and cyanosis Oxygen was given for one-half hour through the mouthpiece He breathed about half through his mouth and half through his nose, thus utilizing about a 60 per cent oxygen mixture The cyanosis distinctly lessened, though it did not wholly disappear The pulse slowed to 108, returning five minutes after administration to its previous rate of from 116 to 120 The respiratory rate also slowed, oscillating between 22 and 26, and remaining at 24 for one-half hour after oxygen The cyanosis largely returned five minutes after oxygen, and seemed completely returned at the end of ten minutes Two hours later, the arterial oxygen saturation was 75.9 per cent, the venous saturation, 60.1 per cent, arterial carbon dioxide content, 62.8 volume per cent, venous carbon dioxide content, 67.0 volume per cent

February 2 (eighth day of disease) Temperature, 102.5 F, pulse, 150, respiration, 44 Patient seemed in agonal state He lay propped up in bed, head thrown back, eyes closed, and in stupor There was a coarse gurgle in his throat during respiration Lips were cyanotic, nail beds were dark deep blue, and backs of hands had a dusky leaden color Breathing was shallow, from 40 to 45 per minute, regular Lungs showed outspoken general edema Besides the coarse bubbling sounds, there were many fine and medium moist râles throughout both chests Pulse small and thready, rate varying between 140 and 160 Oxygen was given through the mouthpiece, patient breathing about 50 per cent oxygen In fifteen minutes, the lip cyanosis had largely disappeared, and the blue color in the nail beds had changed to a light heliotrope shade For one hour and twenty minutes, there was no discernible improvement in pulse or respiration After an hour and thirty-five minutes, the oxygen was stopped for twelve minutes and blood was taken for analysis During this period the cyanosis in the nail beds recurred to the extent before oxygen, although no cyanosis appeared in the lips The arterial saturation was 62.3 per cent, the venous saturation, 53.5 per cent Arterial carbon dioxide content was 60.4 volume per cent The carbon dioxide dissociation curve showed a marked uncompensated carbon dioxide acidosis (The arterial saturation was not determined immediately before oxygen) Oxygen was then continued almost uninterruptedly to make a total duration of two hours and twenty minutes The pulse began to slow after one hour and twenty minutes, and at the end of the administration was 120, of good volume and tension Respiration slowed to 34, seemed deeper, and accompanied by much less dyspnea (See Table 1 for effect on pulse and respiration) The cyanosis was markedly lessened though not completely absent in the nail beds During the administration the patient became wakeful and cognizant of his surroundings, and at the end was mentally alert, said he felt good, and was eager for more oxygen Throughout the day, oxygen was given for about twenty to thirty minutes every hour The cyanosis decreased during the administrations, and recurred after them The pulse and respiration showed no further slowing The coarse gurgle in his throat disappeared, though moist râles were still present everywhere in his lungs That night he was given 15 gm sodium bicarbonate Oxygen was continued through the night, thirty to forty minutes every two hours

February 3 (ninth day of disease) Temperature, 102.4 F, pulse, 124, respiration, 38 Patient continued to manifest improvement Dyspnea and cyanosis distinctly less Cyanosis was now of a light heliotrope shade without

² The work on the carbon dioxide dissociation curve in pneumonia will be reported elsewhere by A. L. Barach, W. N. Woodwell and J. H. Means

oxygen, almost clearing with oxygen. Pulse was of good quality. Oxygen was given fifteen minutes every hour during the day and every two hours at night. Arterial saturation after one of these periods was 82.2 per cent, venous saturation, 78.4 per cent. Arterial carbon dioxide content was 66.7 volume per cent, venous carbon dioxide content, 70 volume per cent. Carbon dioxide dissociation curve was on a higher level than on previous day, and showed no acidosis.

February 4-8. Temperature gradually fell to 99.0 F, pulse and respiration fell more slowly to 110 and 30, respectively. Lungs became resonant throughout with fewer râles. Cyanosis became less marked, but persisted in absence of oxygen. Oxygen was given less often, from twenty minutes every one or two hours to twenty minutes every two or three hours. The cyanosis almost cleared during oxygen, but always reappeared afterward, at times the pulse slowed during oxygen and at times no effect was evident. Slowing was more apt to occur when the patient had not had oxygen for a considerable time previous. February 8, oxygen was given for fifteen minutes by the Haldane apparatus, in which oxygen was supplied through a mask at the rate of four liters per minute, and for a similar period by the mouthpiece rebreathing apparatus, in which about 50 per cent oxygen was breathed by the patient. With the Haldane apparatus

TABLE 1—EFFECT OF OXYGEN IN LOBAR PNEUMONIA WITH PULMONARY EDEMA IN CASE 10

	Pulse	Respiration	Cyanosis
Before oxygen	140-160	40-45	++++
Oxygen for 5 minutes	140	40	++++
Oxygen for 10 minutes	145	40	++++
Oxygen for 15 minutes	145	40	+++
Oxygen for 25 minutes	160	45	+++
Oxygen for 35 minutes	Indeterminate	45	+++
Oxygen for 45 minutes	144	40	++
Oxygen for 55 minutes	140	38	++
Oxygen for 1 hour	142		++
Oxygen for 1 hour 20 minutes	128	30	++
Oxygen for 1 hour 30 minutes	126	40	++
Oxygen for 1 hour 40 minutes	130	38	+++
Oxygen for 2 hours	122	36	++
Oxygen for 2 hours 10 minutes	126	38	++
Oxygen for 2 hours 20 minutes	120	34	++

Cyanosis began to diminish after fifteen minutes' oxygen inhalation, pulse and respiration after one hour and twenty minutes. Patient breathed about 50 per cent oxygen through the mouthpiece. Arterial saturation, 62.4 per cent, venous saturation, 53.5 per cent, arterial carbon dioxide content, 60.4 volume per cent. (Blood tested one hour and forty seven minutes after oxygen was begun, the previous twelve minutes having been without oxygen.)

no effect on the pulse rate was produced. With the mouthpiece rebreathing method, the pulse slowed from 108 to 111 before to 102 after (Table 4). The cyanosis likewise diminished sooner and to a greater extent when the patient was breathing 50 per cent oxygen. The rebreathing bag was kept full by two liters of oxygen per minute so that less oxygen was actually used in this method though offered to the patient in greater concentration. (The Haldane gauge was connected with the rebreathing apparatus for this measurement.)

February 8-15. Patient's temperature again rose and oscillated between 100 and 101 F, pulse, from 100 to 110, respiration, from 27 to 35. Numerous tubercle bacilli were found in the sputum. It was evident that residual process (râles in right chest) was active tuberculosis. A very faint cyanosis persisted in nails. February 14 oxygen was given and electrocardiograms were taken before, during and after oxygen. No change in pulse or electrocardiographic tracings occurred. He proved to be a morphin habitue, and when morphin was finally withheld he left the hospital. He became progressively weaker and more emaciated, and died at home Feb 27 1921.

This patient seemed to have a lobal (pneumococcus) pneumonia in the presence of active pulmonary tuberculosis. He recovered from the pneumonia, but eventually succumbed to the tuberculosis. On admission, he had an arterial (anoxic) anoxemia. The lowered venous saturation did not represent a stagnant circulation, since the relation between arterial and venous oxygen content was normal. (A lowered arterial saturation of necessity reduces the venous saturation, the respiratory exchange taking place at a lower level.) The administration of oxygen by partially relieving the arterial anoxemia brought the venous saturation within normal limits. When edema of the lungs developed, the pulse became extremely rapid and feeble. At that time a true venous (stagnant) anoxemia was undoubtedly present, along with the arterial

TABLE 2—EFFECT OF OXYGEN AS GIVEN BY HALDANE APPARATUS AND AS GIVEN BY MOUTHPIECE REBREATHING METHOD IN CASE 10

	Pulse	Respiration	Cyanosis
Haldane Apparatus 4 liters oxygen per min			
Before oxygen	108-111	28-32	+++
Oxygen for 1 minute	108	28	+++
Oxygen for 2 minutes	108	30	+++
Oxygen for 3 minutes	111	30	++
Oxygen for 4 minutes	108	30	++
Oxygen for 5 minutes	108	30	+
Oxygen for 8 minutes	108	30	++
Oxygen for 10 minutes	108	32	+
Oxygen for 13 minutes	108	30	+
Oxygen for 15 minutes	108	30	+
15 minutes without oxygen	108	27-33	+++
Rebreathing Apparatus 50% oxygen			
Oxygen for 1 minute	111	33	+++
Oxygen for 2 minutes	108	33	++
Oxygen for 3 minutes	107	33	++
Oxygen for 4 minutes	108	33	+
Oxygen for 5 minutes	107	36	+
Oxygen for 8 minutes	102	36	+
Oxygen for 10 minutes	107	33	+
Oxygen for 13 minutes	102	33	+
Oxygen for 15 minutes	102	36	+
10 minutes without oxygen	102-107	33-36	++
15 minutes without oxygen	108	33	+++

With the patient breathing for fifteen minutes through the Haldane apparatus, in which 4 liters of oxygen per minute were added to the inspired air, no effect was produced in the pulse rate. During a period of the same length, with patient breathing about 50 per cent oxygen through the mouthpiece, the pulse distinctly slowed. The cyanosis also decreased more quickly with the mouthpiece method.

anoxemia. Because of what seemed an agonal condition of the patient, oxygen therapy was begun immediately, and no blood was withdrawn for analysis until the pulse had definitely improved. The venous saturation was then almost normal, although an arterial anoxemia still existed. On the following day, the arterial saturation showed a marked increase, though it did not reach normal limits. The effect of a single administration of oxygen accomplished little in this case, the effect of long continued and frequently repeated inhalations of oxygen brought the patient from an obviously moribund condition to one of relative safety, and produced striking improvement in pulse, breathing, color, comfort, and mental condition of the patient, and, apparently, an actual improvement in pulmonary edema.

The fact that the pulse did not slow after oxygen administration for fifteen minutes with the Haldane apparatus and definitely slowed when given with the rebreathing method for the same period is attributable to the higher percentage of oxygen breathed in the latter method. Benedict and Higgins³ found no material change in the pulse rate of normal men when the oxygen inhaled was raised to 40 per cent. A slight, though noticeable, slowing in the pulse occurred when 60 per cent oxygen was breathed, and a very positive slowing when 90 per cent oxygen was administered.

CASE 11 (Table 3)—A M, white female, clerk, age 29 years

Diagnosis—Lobar pneumonia

History—Two days before entry, headache, pains in back and legs, nausea and vomiting began. The same day, patient had a shaking chill, followed by cough, reddish-brown sputum, and pain in right chest.

Physical Examination—Nov 22, 1920 (third day of disease) Temperature, 104.0 F, pulse, 112, respiration, 40. Moderately prostrated and dyspneic. No cyanosis evident. At right posterior chest, below spine of scapula, dullness, bronchial breathing and voice, and crepitant râles were present. Slight dullness at left base. Heart not enlarged, soft systolic murmur at apex. Blood pressure, 120/65. Sputum inoculation, pneumococcus type 11. Blood culture gave a pneumococcus in one flask, second flask, sterile. White blood cells, 19,200, polymorphonuclears, 71 per cent. Blood Wassermann, negative. Urine, sp gr from 1.010 to 1.030, slight trace of albumin, no casts.

Course and Treatment—Routine pneumonia treatment. Nov 24, 1920 (fifth day of illness). Signs on admission unchanged. Patient was moderately prostrated, rational and quiet. Oxygen saturation of arterial blood, 98.8 per cent, venous saturation, 66.0 per cent, arterial carbon dioxide content, 53.5 volume per cent, venous carbon dioxide content, 56.3 volume per cent. Carbon dioxide dissociation curve was within normal limits. Oxygen was given for thirty minutes through the mouthpiece. Nose was occluded most of the time by keeping fingers loosely over nostrils. During the administration, she lay quietly and comfortably, eyes closed most of the period. Face definitely pinkened at the end of the inhalation, without discernible change in hands or nail beds. Directly after, she said she could breathe better and that the pain in the chest was a little better. One hour later she told the nurse she felt wonderfully better and asked when the oxygen was to be given again. The venous saturation five minutes after oxygen was 74.6 per cent, the venous carbon dioxide content, 57 volume per cent. The pulse was 100 before and 100 after oxygen.

November 25 (sixth day of illness). Temperature, 103 F, pulse, 120, respiration, 40. Patient more prostrated than yesterday. Right side of face flushed, remainder colorless. The nails were slightly cyanotic. Respirations were rapid, shallow, with loud grunting expiration. She was quiet and rational, but complained of weakness, and a new sharp pain in the left chest. Lungs showed an extension of bronchial breathing to left posterior chest. Arterial saturation 90.8 per cent, arterial carbon dioxide content, 52.9 volume per cent. Oxygen was given through the mouthpiece, connected with a Douglas bag containing seven liters of an 80 per cent oxygen mixture. After twenty minutes, patient said she felt tired and administration was stopped. (Nose was held occluded during the inhalation). Face showed increased pinkness, lips had better color, and it was doubtful whether the nails had any cyanosis.

³ Benedict, F. G., and Higgins, H. L. Effects on Men at Rest of Breathing Oxygen-Rich Gas Mixtures, *Am J Physiol* 28 1, 1916.

Breathing seemed easier, with less grunting, during and after oxygen. Rate before oxygen, 44, after, 42. Pulse before, from 125 to 130, after, 120. Two hours later she said she felt better and stronger after the inhalation. Oxygen was again given in the evening for twenty-five minutes, this time without holding the nose and connected with tank. She breathed about 80 per cent oxygen. Pulse before oxygen, 128, after oxygen, 120. Five minutes later 124, two hours later, 120, five hours later, 120. Respirations 38 before, 34 after, five minutes later from 36 to 38, two hours later, 32, five hours later (asleep) 30.

November 26 (seventh day of illness) Temperature, 102 F, pulse, 110, respiration, 30. Patient felt better and looked better. Moderately dyspneic, but without expiratory grunt. No discernible cyanosis. Arterial oxygen saturation, 99.7 per cent, arterial carbon dioxide content, 61 volume per cent. Frank signs of consolidation persist over both lower lobes. Oxygen was given through mask for twenty minutes. The soda-lime can was kept in ice water, so that the rebreathing air was cooled. Pulse before oxygen 100, after oxygen 100, one-half hour later 100. Respiration 34 before, after 28, one-half hour later 32. Patient noticed no striking change but thought she could breathe better after the inhalation.

No further oxygen was given. Temperature persisted high, between 101 and 102 F for nine more days, reaching normal on the 16th day of illness. She left the hospital three weeks later, lungs clear. Patient spoke repeatedly of the relief oxygen afforded her.

On the fifth day of illness, patient had no anoxemia. The administration of oxygen produced distinct subjective improvement but no change in pulse or respiratory rate. The venous oxygen saturation was raised. On the sixth day an extension of the pneumonic process occurred to the left lung, and an arterial anoxemia was present. Oxygen was given twice that day. Pulse was distinctly slowed after each administration, respiratory rate after the second. Subjective relief was again experienced and the following morning no anoxemia was present. Oxygen was again given but without effect on pulse and with slight slowing of respiration. In this case the slowing of the pulse occurred when the patient was anoxemic and not when the blood was normally saturated.

CASE 12 (Table 3)—D. H., white, male, chauffeur, age 21 years.

Diagnosis—Lobar pneumonia. Pulmonary edema.

History—For six days patient had a slight cold with cough. On the sixth day he was awakened from sleep by a severe pain in right side, felt chilly, began to cough frequently and raised rusty sputum. Entered the hospital the same day.

Physical Examination—Feb. 24, 1921 (first day of disease) Temperature, 103.4 F, pulse, 128, respiration, 48. Moderate dyspnea. Very slight cyanosis of nail beds. Lungs showed dullness and a friction rub at right base. Heart without enlargement, murmurs, or irregularity. Pulse full and bounding. Urine negative. Blood culture sterile. Sputum inoculation showed pneumococcus type I. White blood cells 22,000, polymorphonuclears 88 per cent.

Course and Treatment—Routine pneumonia treatment. Serum therapy. On the third day of illness, 100 cc of pneumococcus I serum was given, on the fourth day, 100 cc of serum was given in the morning and 100 cc in the evening. No improvement resulted in the prostration of the patient, the temperature, pulse, or respiration, and on the morning of the fifth day he was strikingly worse.

February 28 (fifth day of disease) Temperature, 103.5 F, pulse, from 140 to 150, respiration, from 35 to 40. Marked prostration and dyspnea. Patient stuporous. Finger-nails deeply cyanotic, lips and ears a bluish-red color. Signs of consolidation had extended to left lower lobe, and many coarse moist râles were present throughout both lungs. White blood cells had dropped to 11,000. Oxygen saturation of the venous blood was 63.5 per cent, venous carbon dioxide content, 51.7 volume per cent. Arterial blood was not tested, but the deep cyanosis was of necessity due to a marked lowering of the arterial saturation, since no venous anoxemia was present. Oxygen administration was attempted with the Haldane apparatus, but the patient struggled against the mask, and it had to be given up. The mouthpiece was substituted, and he breathed through it in comfort, using about 40 per cent oxygen. His condition steadily improved. At the end of two hours continuous inhalation his color, pulse, breathing, and mental reaction showed decided betterment. Pulse rate before oxygen, from 136 to 140, after two hours, from 124 to 128. Respiration, 30 before, 28 after. Cyanosis became markedly diminished, although it did not wholly disappear. Oxygen was given for six more times during the day and evening, each time for about half an hour. The pulse during oxygen was usually 128, in the periods between oxygen, 132. The cyanosis reappeared during the intervals between oxygen, though not to the extent present in the morning, becoming diminished correspondingly during the inhalation. Serum was given that afternoon, at midnight, and the next morning, 100 cc each time.

March 1 (sixth day of disease) Temperature, from 102.5 to 100.2 F, pulse from 128 to 100, respiration, 30. Patient manifested continued improvement. Prostration, dyspnea and cyanosis were much lessened. Oxygen had been given eight times through the night up to twelve o'clock noon, in periods varying from twenty minutes to one hour. Blood analysis then showed an arterial saturation of 88.9 per cent and a venous saturation of 64.5 per cent. Arterial carbon dioxide content, 53 volume per cent, venous carbon dioxide content, 55.5 volume per cent. Oxygen was given four times more during the day and evening. The temperature began to fall at midnight of the fifth day, and gradually fell during the sixth day, reaching 100.5 F at midnight, apparently a slow crisis.

March 2-3 (seventh and eighth days of disease) Temperature, 100.0 to 101.0 F, pulse 110 to 100, respiration, 28 to 24. Although the crisis was over, patient appeared dull and weak and felt tired. Slight but definite cyanosis persisted in the finger-nails. Oxygen was given four times March 2 and once March 3, each time for about one-half hour. March 3, the arterial saturation after oxygen was 93 per cent. The coarse moist râles disappeared anteriorly but were still numerous at both bases posteriorly. The cyanosis disappeared completely three days later, the temperature became flat on the following day, and the patient left the hospital ten days later feeling well and with lungs clear.

In this patient the development of pulmonary edema on the fifth day of the disease seemed to indicate a fatal outcome. The continued inhalation of oxygen produced a striking improvement in the clinical picture and greatly relieved the arterial anoxemia. Slight lowering of the arterial saturation persisted after oxygen administration and for five days after the crisis. Oxygen inhalation in this case relieved a condition of acute oxygen want which might otherwise have proved disastrous.

CASE 13 (Table 3)—A. A., white male, carpenter, 26 years

Diagnosis—Lobar pneumonia

History—Five days before admission, patient had a chill, and since then has felt hot, feverish and weak. The next day he developed cough and blood tinged sputum. For past three days he has had a sharp pain in right chest, worse on inspiration.

Physical Examination—Jan 5 1921 (fifth day of disease) Temperature, 104 F, pulse, 100, respiration, 36. Moderate dyspnea. Slight cyanosis of finger-nails. Dulness, bronchial breathing, and crepitant râles over right posterior chest below angle of scapula. Dulness and coarse moist râles at left base. Heart sounds faintly heard, no murmurs. Pulse full and not rapid. Premature ventricular contraction occurred once in every six or ten beats (electrocardiogram). Moderate jaundice of sclera and skin. Urine, sp. gr. from 1.018 to 1.034, occasional slight trace of albumin and occasional cast, bile for five days. White blood cells, 15,000, polymorphonuclears, 83 per cent. Blood culture sterile. Blood Wassermann negative. Sputum inoculation gave a pneumococcus type I. Arterial oxygen saturation, 92.4 per cent, arterial carbon dioxide content, 42 volume per cent. Carbon dioxide dissociation curve showed an uncompensated carbon dioxide acidosis.

Course and Treatment—Routine pneumonia treatment. No serum therapy. Digitalis leaves, 1½ grains, twice a day, January 5-7, 1921.

January 6 (sixth day of disease) Temperature, from 104 to 105 F, pulse, from 100 to 130, respiration, from 30 to 45. Patient little changed. Oxygen was commenced with the mouthpiece, but patient inhaled mostly through his nose and blew out through the tube. He did not understand English, and procedure could not be explained to him. The mask was then adjusted connected to the rebreathing bag, and this he took fairly well, duration of administration, twenty minutes. Arterial saturation before oxygen 94.3 per cent, four minutes after, 98 per cent, venous saturation before, 79.5 per cent, arterial carbon dioxide content before, 44.3 volume per cent, after, 44.8 volume per cent. Carbon dioxide dissociation curve gave evidence of a carbon dioxide acidosis. Immediately after oxygen was begun, a striking slowing of the pulse occurred, from 116 to 92. When oxygen was temporarily stopped, it rapidly mounted to its former rate. This process could be duplicated at will, and it was found that the following occurrence was fairly constant. After ten seconds oxygen, the pulse rate was 108, after twenty-five seconds, 92 or 88, after oxygen was withdrawn for fifteen seconds, the pulse had risen to 108, after thirty seconds, to 116. As long as the inhalation was continued the pulse was from 88 to 92. In addition, the premature contractions which occurred every six to eight beats previously were absent during oxygen inhalation. The respirations remained 44 throughout. The finger-nails showed no certain change in color. Subjectively, the patient noticed no change.

January 7 (seventh day of disease) Temperature, from 104.2 to 105.4 F, pulse, from 110 to 128, respiration, from 35 to 44. Condition unchanged. It was attempted to give oxygen and follow cardiac changes with the electrocardiograph. Patient, however, struggled against the mask. Oxygen given for fifteen minutes showed no change in pulse rate, electrocardiographic tracings, or in respiratory rate. The oxygen saturation of the arterial blood before the inhalation was 96.5 per cent, arterial carbon dioxide content, 45.3 volume per cent.

Two days later crisis occurred and patient speedily recovered.

January 18. Patient well. Arterial saturation 96.5 per cent, arterial carbon dioxide content, 50.6 volume per cent. Carbon dioxide dissociation curve showed no acidosis.

In this case a very slight arterial anoxemia was fully corrected by the inhalation of oxygen. During the first administration, a remarkable slowing of the pulse occurred, lasting only as long as oxygen was given. It is possible that this was not a direct oxygen effect but of vagal origin due to the increased depth of breathing. The arterial blood was normally saturated on the day following the inhalation. No subjective improvement occurred.

CASE 14 (Table 3)—L. M., white, male, salesman, age 42 years

Diagnosis—Lobar pneumonia, empyema, pyopneumothorax, chronic alcoholism (necropsy)

History—Whiskey for years. One pint daily in past year. Four days ago following exposure to wet he had a shaking chill. On the next day he had generalized pains, cough, and sputum. Two days ago he began to have pain in left side and marked dyspnea.

Physical Examination—Nov. 27, 1920 (fourth day of disease). Temperature, 105 F., pulse, 140, respiration, 45. Marked dyspnea with grunting expiration, extreme restlessness and pleuritic pain. Face and neck deeply flushed. Cheeks had a bluish tinge. Lips and finger-nails deeply cyanotic. Dulness, bronchial breathing and many medium moist râles over left lower lobe. Crepitant râles at right base. Heart sounds rapid, without murmurs. Pulse of poor quality, rate 140. Slight distention of abdomen. Urine, sp. gr., from 1.028 to 1.038, slight trace of albumin, frequent granular casts. Blood Wassermann, strongly positive. White blood cells 20,800, polymorphonuclears, 90 per cent. Blood culture, sterile.

Course and Treatment—Routine pneumonia treatment. Chest swathe worn throughout illness.

November 29, 1920 (sixth day of disease). Temperature, 103 F., pulse, 138, respiration, 44. Condition essentially the same as on admission. Oxygen was given for twenty minutes through a loosely fitting mask. Patient was uncomfortable, and inhalation had to be frequently interrupted. Respiration became deeper and slower during administration, rate 36 before, from 30 to 32 during, 36 after. Pulse, from 140 to 144 before and during administration. Lips seemed to have a trifle better color after oxygen but no change was observed in the cyanosis of the nails. Oxygen saturation of the venous blood before the inhalation 52.6 per cent, after, 56 per cent. After oxygen breathing seemed to have less of the grunting character. Patient thought he could breathe easier after the oxygen but was not enthusiastic. In the evening oxygen was given through the mouthpiece for twenty-five minutes. He breathed easily and comfortably, using about 60 per cent oxygen. Respiration became deeper and slower, rate before, 44, during, from 26 to 34, five minutes after, 44. Pulse before, 155, during, 144, five minutes after, 150. It was not possible to decide about any change in color.

November 30 (seventh day of disease). Temperature, 102 F., pulse, 135, respiration, 40. Patient in great distress because of pleuritic pain and dyspnea. Lips were very blue. Finger-nails, backs of hands, and ears were slightly cyanotic. Arterial oxygen saturation, 74.4 per cent, venous saturation, 54.8 per cent, arterial carbon dioxide content, 47 volume per cent. Carbon dioxide dissociation curve showed an uncompensated carbon dioxide acidosis. Oxygen was given through the mouthpiece for twenty minutes. Lips seemed a trifle less cyanotic, nail-bed cyanosis unchanged. Pulse before, from 152 to 155, during, 148, after, from 150 to 155. Respiration, from 48 to 50 before, from 36 to 48 during, from 44 to 48 after. Breathing seemed less arduous after oxygen, and patient said he felt more comfortable. Oxygen was given three times more that day, in fifteen minute periods. He complained of feeling tired after the last inhalation.

December 1 (eighth day of disease) Temperature, from 101 to 103 F, pulse, 150, respiration, 40 Dyspnea and pleuritic pain continued unabated Cyanosis of nails seemed less marked, lips still deeply cyanosed Venous saturation 32.2 per cent Oxygen was given for fifteen minutes through the mouthpiece eight times during the day The pulse slowed slightly each time, returning to previous rate when the inhalation was stopped The respiratory rate remained unaffected, but the breathing seemed deeper Color and comfort little changed

December 2 (ninth day of disease) Temperature, 101 F, pulse, 140, respiration, 36 Dyspnea and cyanosis less marked Pulse still rapid, compressible, poor quality Oxygen was given as on previous day for three times in the morning and three times in the afternoon, and blood tested at 1:30 p. m. Arterial saturation, 91.6 per cent, venous saturation, 36 per cent, arterial carbon dioxide content, 47.2 volume per cent, venous carbon dioxide content, 53.7 volume per cent Carbon dioxide dissociation curve showed no acidosis

December 3 (tenth day of disease) Temperature, from 102 to 104 F, pulse, 148, respiration, 35 Cyanosis diminished in lips and nail beds General condition about the same Oxygen given for fifteen minutes twice in the morning and twice in the afternoon and blood tested at noon Venous saturation, 46.1 per cent

December 4-5 (eleventh and twelfth days of disease) Temperature, from 99.5 to 104 F, pulse, 140, respiration, from 30 to 40 Condition was little changed No oxygen was given Cyanosis did not increase

December 6 (thirteenth day of disease) Temperature 105 F, pulse, 140, respiration, 40 There was slight cyanosis of the lips and barely perceptible cyanosis of nail beds Arterial saturation, 92.7 per cent, venous saturation, 57.8 per cent, arterial carbon dioxide content, 40.6 volume per cent, venous carbon dioxide content, 49.2 volume per cent Oxygen was given through a mask with valve attachment for twenty minutes The venous saturation immediately after was 58 per cent, the venous carbon dioxide content, 50 volume per cent The respiration before the administration was 40 per minute, during the administration from 32 to 36, and five minutes after, 40 The pulse varied between 140 and 144, and was unchanged by the treatment There seemed to be a very slight diminution of the lip cyanosis, but no other change in color The patient said he felt no relief during the inhalation, but directly afterward felt fine One-half hour later chest puncture revealed purulent fluid, the culture of which subsequently showed staphylococcus, streptococcus, and gram negative bacilli That night the patient died

In the beginning, the patient had a severe arterial (anoxic) anoxemia, with only a relative lowering of the venous saturation In the middle of his course, a true venous (stagnant) anoxemia developed and became the dominant feature of his illness This paralleled a pulse of a persistently poor quality and rapid rate Toward the end, the arterial anoxemia became markedly diminished, and later the venous anoxemia was much reduced The patient was obstreperous during his entire illness He resisted taking the mask, but took the mouthpiece for short periods very well, complaining, however, of long periods Inhalation of oxygen, in this case, caused no immediate increase in oxygen saturation of the venous blood The arterial saturation increased under oxygen administration, but it is possible that much of this improvement was due to changes in the lungs No arterial determinations were done immediately before and after an inhalation

After oxygen had been given at repeated intervals for two days, an increase from 74.4 to 91.6 per cent occurred. The cyanosis showed only a very slight to a doubtful clearing during the administration, though in the above two days it gradually diminished. It is possible that the venous anoxemia present at this time was responsible for the persistence of the cyanosis during the administration, and that the continued oxygen inhalations did have an influence in the raising of the arterial saturation. However, this case was exceptional in the fact that oxygen inhalation (even 80 per cent oxygen) had so little effect on the cyanosis. To some extent this can be ascribed to the fact that there was a marked venous anoxemia due to the insufficient circulation and in part to an arterial anoxemia due to a blood flow through an area of consolidation. The pulse was usually temporarily slowed. The respiration likewise, was as a rule, slowed. The patient said that he felt better after the treatments, but that it did not last. His final fatal outcome was attended with only a slight anoxemia both arterial and venous, and was due to empyema sepsis. The carbon dioxide dissociation curve showed an acidosis which disappeared with the diminution in arterial anoxemia.

CASE 15 (Table 3)—T. B., white, male, longshoreman, age 47 years

Diagnosis—Lobar pneumonia, empyema

History—Four days before admission he had a sudden chill followed by inspiratory pain in left chest, feverishness and weakness. Two days later he began to cough, spit up blood tinged sputum and became for a time delirious.

Physical Examination—Nov. 28, 1920 (fourth day of disease). Temperature 104 F, pulse, 100, respiration, 40. Dyspnea without cyanosis. Over lower half of left chest anteriorly and posteriorly there were dullness, bronchial voice and breath sounds with few crepitant râles. At right base posteriorly, dullness diminished, breathing and crepitant râles present. Heart sounds regular, good quality, no murmurs. Pulse full and not rapid. Sputum inoculation, pneumococcus I, blood culture, pneumococcus I. White blood cells 16,400, polymorphonuclears, 68 per cent. Urine, sp. gr., from 1.008 to 1.025, trace of albumin. Blood Wassermann negative.

Course and Treatment—Routine pneumonia treatment. No serum therapy.

December 3, 1920, (ninth day of disease). Elevated temperature, pulse and respiration and moderate prostration continued. Thoracentesis of left chest revealed 42 c.c. cloudy yellow fluid, from which pneumococcus I in pure culture was recovered. Aspiration of left chest done daily thereafter and small amounts of opaque yellow fluid removed.

December 7 (thirteenth day of disease). Temperature had fallen to 100.5 F. Patient appeared and felt weak. Cheeks were deeply sunken. Face had a slight reddish flush, without actual cyanosis. Fingers were pale. Lung signs same as on admission except for egophony at left base. Pulse, 100, respiration, 25. Oxygen saturation of arterial blood 88.9, arterial carbon dioxide content, 49 volume per cent.

December 8 (fourteenth day of disease). Temperature, 99 F. Appearance same as yesterday. Said he felt dazed. Oxygen was given through mouthpiece for twenty minutes, patient using about an 80 per cent oxygen mixture. Felt perfectly comfortable but noticed no change in his condition. No cyanosis was evident previous to administration and no increased pinkness occurred after it.

Respiratory rate 24 before and after, but increased depth seemed to be present. Pulse 96 before, 92 after, 92 one-half hour later. Arterial saturation immediately before 85.5 per cent, immediately after, 100 per cent, arterial carbon dioxide content 47 volume per cent.

December 9 (fifteenth day of disease). Temperature 99 F. Appearance unchanged. Felt "pretty good, but weak." Arterial saturation, 96.6 per cent, venous saturation, 88.2 per cent. Oxygen given as on previous day. Pulse 86 before, 84 after, respiration 22 before and after. No change subjectively. Arterial saturation at end of oxygen 98.7 per cent, venous saturation 88.9 per cent, arterial carbon dioxide content, 50 volume per cent, unchanged by administration. No further oxygen was administered. Patient was operated on two days later, simple thoracotomy with insertion of catheter, and again ten days later, rib resection. He made a good recovery. Discharged Jan. 9, 1921, with very slight discharge from operative site.

In this patient empyema was present for five days before oxygen was administered. Daily aspiration reduced the temperature, but failed to relieve the prostration. An arterial anoxemia¹ was demonstrated for the two days preceding the administration, was completely removed by inhalation of oxygen for one-half hour, and continued absent on the following day. Beyond the slowing of the pulse and the deeper breathing no other clinical effects were observed. It cannot be said definitely that the single administration of oxygen caused the oxygen of the arterial blood to remain elevated. Actual changes in the lungs were perhaps the cause. However, the increased depth of respiration may have persisted and accomplished complete aeration of the lung. That a vicious circle of shallow breathing may itself cause anoxemia and be completely and permanently relieved by oxygen inhalation has been emphasized by Haldane and commented upon in the previous paper. This condition of affairs may well be present in weakened states following pneumonia, especially in the course of a disease where shallow breathing is encouraged by long-standing pleurisy. Subjectively, he felt no improvement during or after the administration.

CASE 16 (Table 3)—L. N., white, female, housewife, age 53 years.

Diagnosis—Bronchopneumonia, asthma.

History—Attacks of tightness in chest, cough and sputum for thirty years. Three weeks before admission developed a head cold, one week later a cough, and six days before entry pains in right chest, prostration and dyspnea.

Physical Examination—Temperature, 102 F, pulse, 120, respiration 35. Moderate dyspnea. Slight cyanosis of mucous membranes. Lungs contain sibilant and sonorous râles scattered throughout. Over right lower lobe posteriorly, increased voice and tactile fremitus present with fine crackling râles. Heart not enlarged, no murmurs. Blood culture sterile. Pneumococcus I in sputum. White blood cells 20,800, polymorphonuclears, 86 per cent. Blood Wassermann negative. Urine negative.

Course and Treatment—Routine pneumonia treatment. On the first two days, the temperature oscillated between 100 and 102 F, and on the third day (and thereafter) it remained below 100.5 F.

January 17, 1921 (third day in hospital). Patient was quite comfortable and complained only of shortness of breath. Dyspnea was moderate. No cyanosis evident. Oxygen was administered through the mouthpiece for twenty minutes. She breathed comfortably throughout, using about 70 per cent oxygen, and at

the end said the oxygen had a "soothing" effect. Saturation of the arterial blood directly before was 96.5 per cent, directly after, 98 per cent, arterial carbon dioxide content before, 50 volume per cent, after, 50.5 volume per cent. Pulse, 96 before, 93 after. Respiratory rate, 28 before, 28 after. No change in color of skin or mucous membranes.

January 22. Temperature, pulse and respiration normal. Patient without symptoms.

This case is an example of a patient without anoxemia at the time of administration of oxygen. The arterial saturation was raised, the pulse slightly lowered, the respiratory rate unaltered. Subjectively, she was apparently much helped. The case itself was atypical in onset and course from the standpoint of a pneumococcus pneumonia.

CASE 17 (Table 3) — F. D., white, male, barber, age 23 years.

Diagnosis — Septicemia, bronchopneumonia, erysipelas.

History — Present illness began two weeks before admission with nervousness, vomiting, weakness and insomnia. For the week before admission he had fever, was at times irrational, and at times stuporous.

Physical Examination — December 28, 1920. Temperature, 104 F, pulse, 140, respiration, 40. Stupor and muscular twitchings. Respirations deep and rapid, rate 40. Heart not enlarged, no murmurs. Knee jerks active and equal. Lungs clear on day of admission. On following day, dullness, bronchial breathing, increased voice and tactile fremitus at left base posteriorly. Blood Wassermann negative. Two blood cultures negative. White blood cells, 11,000, polymorphonuclears, 70 per cent. Two Widal's negative. Spinal fluid, normal pressure, negative for globulin, twenty-two cells, eighteen lymphocytes, four polymorphonuclear leukocytes. Sputum, pneumococcus type IV. Urine, sp. gr., from 1.020 to 1.030, slight trace of albumin.

Course and Treatment — Typhoid precautions, forced fluids, soft solids.

Jan. 3, 1921. Temperature, 103 F, pulse, 130, respiration, 40. Patient had been in hospital seven days without any essential change in condition. Respirations were rapid, regular, and deep, without grunting, and entirely through the nose. Lips were a dark deep red. No cyanosis of face or fingers. His hands moved restlessly and twitched at the bed clothes. Oxygen saturation of the arterial blood, 98.9 per cent, of the venous blood 82.3 per cent, arterial carbon dioxide content, 42 volume per cent. Oxygen was administered through the mouthpiece for one-half hour, patient inhaling about 80 per cent oxygen. He breathed deeply as before, but quietly and comfortably, eyes closed most of the time. At the end, there was a little increased pinkness under the eyelids. Pulse varied between 129 and 135 before oxygen, 126 and 129 during oxygen, 114 and 120 for the three hours following. Respiratory rate, 40 before and after.

January 4. Patient was stuporous, otherwise unchanged. Arterial saturation 93.9 per cent, venous saturation 77.1 per cent, arterial carbon dioxide content, 42.4 volume per cent. Carbon dioxide dissociation curve showed an uncompensated acidosis. No further oxygen was administered. Sodium bicarbonate was given, correcting the acidosis and ameliorating his stupor and hyperpnea. Arterial saturation on the following day 97.7 per cent, venous saturation 85.2 per cent, arterial carbon dioxide content, 44.5 volume per cent. Carbon dioxide dissociation curve showed a slight alkalosis. He failed to improve however and five days later, having developed a terminal erysipelas, died.

The clinical diagnosis was septicemia with metastatic bronchopneumonia. The arterial saturation was normal, the venous saturation above normal. (In several cases of acidosis of varying causes the

oxygen saturation of the venous blood has been found higher than normal and is probably explained by the hyperpnea) Oxygen was easily and effectively given but without any definite changes in his condition This was to be expected in the face of high normal values of oxygen saturation The dyspnea and hyperpnea are partly accounted for by an acidosis, but since some dyspnea and hyperpnea were present even after this was corrected one must look further for a toxic factor, presumably acting on the respiratory center

CASE 18 (Table 3) —E A, white, male, machine finisher, 53 years Admitted March 4, 1921

Diagnosis—Lobar pneumonia

History—Eight days before entry, he was seized with a severe chill and pain in right side On the following day he developed cough and rusty sputum Condition grew steadily worse and he became cyanotic

Physical Examination—March 4, 1921 (eighth day of disease) Temperature, from 102.5 to 105 F, pulse, 128, respiration, 35 Stuporous, irrational, dyspneic Low gurgling sounds during respiration Finger-nails, a grayish dusky color, ears slightly cyanotic Intermittent hiccough Dulness, bronchial breathing, and many coarse moist râles over right upper and middle lobes, and left upper and left lower lobe Heart slightly enlarged, without murmurs or irregularity Pulse easily compressible, good volume, rate 128 Urine, sp gr, from 1.010 to 1.020, occasional slight trace of albumin Blood Wassermann negative White blood cells, 16,600, polymorphonuclears, 90 per cent Sputum inoculation gave a pneumococcus type I

Course and Treatment—Routine pneumonia treatment No digitalis

March 4, 1921 (eighth day of disease) Arterial saturation, 76.8 per cent, venous saturation, 71.5 per cent, arterial carbon dioxide content, 49.1 volume per cent, venous carbon dioxide content, 51.2 volume per cent Oxygen was given through the Haldane apparatus, rate from 3 to 4 liters per minute, for two hours in the morning, three hours in the afternoon, and six hours in the night Pulse was 130 before, 122 after oxygen had been given one and one-half hours Respiration, 32 before, 28 after one and one-half hours Cyanosis cleared No further reduction in pulse or respiration occurred

March 5 (ninth day of disease) Temperature, from 102 to 103.5 F, pulse, 120, respiration, 35 Condition seemed definitely improved No gurgling sounds during respiration Oxygen was given for two hours in the morning, and blood taken for analysis at the end, while patient was still breathing through mask Arterial saturation, 100 per cent, venous saturation, 79.1 per cent, arterial carbon dioxide content, 49.5 volume per cent Pulse 120 before, 111 after Respiration, 35 before, 36 after Oxygen given for two hours in the afternoon, and six hours in the night

March 6 (tenth day of disease) Temperature from 101 to 102.5 F, pulse, 102, respiration, 28 General condition, little changed Patient was still irrational, though less stuporous Slight cyanotic tinge to finger-nails in the morning (Had not had oxygen for one hour) Oxygen was given for eight hours in the day and night, usually in one-hour periods

March 7, (eleventh day of disease) Temperature, 98.6 F, pulse, 90, respiration from 22 to 25 Crisis occurred during the night Patient was still irrational Hiccup continued Lungs showed many coarse moist râles over both chests posteriorly There was still a slight cyanotic tinge in the absence of oxygen During the following five days, oxygen was given for one hour, three times a day, at the end of which time the finger-nails possessed no cyanosis in the absence of oxygen Patient was still slightly irrational, but gradually

TABLE 3—EFFECT OF OXYGEN THERAPY ON THE BLOOD GASES IN TWELVE CASES OF PNEUMONIA

Case No.	Diagnosis	Date	Oxygen Inhalation	Oxygen Content		Oxygen Saturation		Hemoglobin Calculated from Oxygen Capacity	Carbon dioxide Content		Remarks
				Arterial Vol %	Venous Vol %	Arterial %	Venous %		Arterial Vol %	Venous Vol %	
10	Lobar pneumonia, chronic pulmonary tuberculosis, pulmonary edema	1/31/21 2/1/21 2/2/21	No oxygen 2 hrs after oxygen 12 min after oxygen	15.69 15.72 13.89	10.29 12.43 11.92	77.2 75.9 62.3	70.2 69.1 53.5	20.30 20.74 22.30	49.5 62.8 60.4	53.1 67.0 60.2	Cyanosis +++ Cyanosis +++ Developed pulmonary edema, cyanosis +++ Gurgling respiration absent, cyanosis +
11	Lobar pneumonia	2/3/21	Oxygen day and night	17.02	16.22	82.2	78.4	20.75	66.7	70.0	R
12	Lobar pneumonia, pulmonary edema	11/24/20	Before oxygen	18.60	12.42	98.8	66.0	18.81	53.5	56.3	R
		Oxygen for 30 min	18.21	14.03	90.8	74.6	101.8	52.9	56.3	57.0	Consolidation extended to L L
		Before oxygen	18.04	13.53	99.7	63.5	115.2	61.0	51.7	51.7	Pulmonary edema, cyanosis +++
		Before oxygen	16.49	11.95	88.9	64.5	100.1	53.0	55.5	55.5	Cyanosis +
13	Lobar pneumonia	3/3/21	Oxygen for 30 min	17.65	10.22	93.0	74.4	19.00	42.0	45.0	R
		Before oxygen	19.64	14.72	94.3	79.5	115.0	44.3	45.0	45.0	Cyanosis +
		1/5/21	Before oxygen	17.48	14.14	93.0	79.5	100.1	44.3	45.0	Cyanosis +
		1/6/21	Before oxygen	15.76	10.22	90.5	74.4	18.81	42.0	45.0	Cyanosis +
14	Lobar pneumonia, emphysema, chronic alcoholism	1/7/21	No oxygen	16.54	9.63	90.5	53.6	17.14	50.6	50.6	D
		Before oxygen	18.98	11.83	92.7	57.8	110.5	40.6	49.2	50.0	Cyanosis +, day of death
		Before oxygen	15.04	10.22	88.9	58.0	100.1	49.0	50.0	50.0	Empyema present before treatment
		Before oxygen	13.24	9.00	84.5	58.0	83.8	47.0	50.0	50.0	No cyanosis
15	Lobar pneumonia, emphysema	12/7/20	No oxygen	13.24	9.00	84.5	58.0	83.8	47.0	50.0	R
		Before oxygen	16.10	12.58	100.0	81.2	100.0	40.6	49.2	50.0	Cyanosis +
		Before oxygen	14.95	13.43	96.6	83.2	100.0	40.6	49.2	50.0	Cyanosis +
		Before oxygen	15.28	13.43	96.6	83.2	100.0	40.6	49.2	50.0	Cyanosis +
16	Bronchopneumonia	1/7/21	Before oxygen	12.05	9.63	90.5	53.6	17.14	50.6	50.6	D
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	No cyanosis
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	No cyanosis
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	No cyanosis
17	Septicemia, broncho pneumonia	1/3/21	Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	D
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	No cyanosis
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	No cyanosis
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	No cyanosis
18	Lobar pneumonia, pulmonary edema	1/4/21	Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	R
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	Pulmonary edema, cyanosis +++
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	Cyanosis +
		Before oxygen	12.24	12.05	90.5	53.6	17.14	50.6	50.6	50.6	Cyanosis +
19	Lobar pneumonia, acute pulmonary tuberculosis	1/5/21	No oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	R
		Before oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	51.2	Cyanosis +
		Before oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	51.2	Cyanosis +
		Before oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	51.2	Cyanosis +
20	Lobar pneumonia	3/5/21	Oxygen for 13 hrs	18.84	14.73	100.0	79.1	18.65	49.5	41.2	R
		Before oxygen	18.84	14.73	100.0	79.1	18.65	49.5	41.2	41.2	Cyanosis +
		Before oxygen	18.84	14.73	100.0	79.1	18.65	49.5	41.2	41.2	Cyanosis +
		Before oxygen	18.84	14.73	100.0	79.1	18.65	49.5	41.2	41.2	Cyanosis +
21	Lobar pneumonia	2/15/21	2 1/2 hrs after oxygen	19.36	13.08	92.5	64.1	20.40	36.6	41.4	R
		Before oxygen	19.36	13.08	92.5	64.1	20.40	36.6	41.4	41.4	Cyanosis +
		Before oxygen	19.36	13.08	92.5	64.1	20.40	36.6	41.4	41.4	Cyanosis +
		Before oxygen	19.36	13.08	92.5	64.1	20.40	36.6	41.4	41.4	Cyanosis +
22	Bronchopneumonia	3/11/21	No oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	R
		Before oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	51.2	Cyanosis +
		Before oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	51.2	Cyanosis +
		Before oxygen	12.91	12.03	76.8	71.5	16.83	49.1	51.2	51.2	Cyanosis +
23	Chronic nephritis, uremia, bronchopneumonia, pulmonary edema	12/11/20	Not treated	20.24	14.73	91.9	65.9	22.3	37.8	51.6	D
		Before oxygen	20.24	14.73	91.9	65.9	22.3	37.8	51.6	51.6	Cyanosis +
		Before oxygen	20.24	14.73	91.9	65.9	22.3	37.8	51.6	51.6	Cyanosis +
		Before oxygen	20.24	14.73	91.9	65.9	22.3	37.8	51.6	51.6	Cyanosis +
24	Chronic nephritis, uremia, bronchopneumonia, pulmonary edema	12/13/20	Not treated	16.74	14.92	78.6	65.9	21.30	35.4	44.8	D
		Before oxygen	16.74	14.92	78.6	65.9	21.30	35.4	44.8	44.8	Cyanosis +
		Before oxygen	16.74	14.92	78.6	65.9	21.30	35.4	44.8	44.8	Cyanosis +
		Before oxygen	16.74	14.92	78.6	65.9	21.30	35.4	44.8	44.8	Cyanosis +

* The arterial content was 0.6 volume per cent. higher than the capacity. This probably represents an excess of oxygen in physical solution.

+ The arterial content was 0.6 volume per cent. higher than the capacity. This probably represents an excess of oxygen in physical solution.

regained his normal mental state in the next four days. Lung signs largely cleared. He left the hospital twenty days after the crisis, feeling completely well.

This patient had a severe arterial (anoxic) anoxemia with a normal venous saturation. The diminished arterial saturation can be accounted for by the marked amount of edema that was present over the consolidated areas. The difference between the arterial and venous saturation was even less than normal, indicating presumably an efficient blood flow attempting to compensate for the diminished oxygen in the arterial blood. The administration of oxygen with the Haldane apparatus for thirteen out of twenty-six hours raised the arterial saturation to the normal, elevated slightly the venous saturation, and resulted in definite clinical improvement. For three days oxygen was administered from eight to eleven hours a day, at the end of which time patient had a crisis and thereby passed from an extremely critical condition to one of comparative safety. It seemed that the administration of oxygen in this very sick patient averted a fatal outcome by keeping him largely free from anoxemia for the three days preceding the crisis.

CASE 19 (Table 3)—M. D., white, school girl, 18 years. Admitted March 11, 1921.

Diagnosis—Lobar pneumonia, acute pulmonary tuberculosis.

History—Chronic cough for 4 years. Pleurisy with effusion one year before entry. Six days before entry, headache and vomiting began, followed in next two days by cough, rusty sputum, and pain in right side.

Physical Examination—March 11, 1921 (sixth day of disease). Temperature, 104 F., pulse, 122, respiration, 40. Dyspneic grunting respiration. Moderate cyanosis of nails and mucous membranes. Signs of consolidation over left lower lobe, with few consonating râles. Heart without enlargement, irregularity, or murmurs. Pulse full and of good tension. Urine, sp. gr. from 1.013 to 1.028, slight trace of albumin. Blood Wassermann, negative. White blood cells, 19,000, polymorphonuclears, 78 per cent. Sputum, pneumococcus II and acid-fast bacilli. Blood culture sterile.

Course and Treatment—Routine pneumonia treatment. Digitalis leaves 1½ grains, twice a day from March 12 to March 21.

March 16, 1921 (eleventh day of disease). Temperature 103.4 F., pulse 120, respiration, 35. Patient continued to be markedly prostrated. Signs of consolidation had spread to right lower lobe. Very few râles. Arterial saturation 87.5 per cent, venous saturation, 78.6 per cent, arterial carbon dioxide content, 40.4 volume per cent, venous carbon dioxide content, 41.2 volume per cent. Carbon dioxide dissociation curve showed no acidosis. Oxygen was given through mouthpiece for fifteen minutes, patient breathing about 40 per cent oxygen. Slight cyanotic tinge to nails seemed less marked after oxygen. Pulse before, 120, after, 110, five minutes later, 112, fifteen minutes later, 108, forty-five minutes later, 116, one hour later, 120. Respiration from 34 to 36, unchanged throughout.

March 17 (twelfth day of disease). Temperature, 103.2 F., pulse, 104, respiration, 34. Condition little changed. Oxygen given for twenty-five minutes. Pulse before, 104, after, from 96 to 100. Respiration 34, unchanged. Very slight cyanotic tinge in the nails seemed diminished after oxygen. On both occasions patient thought oxygen helped her a little by making her breathing easier.

Patient remained in the hospital twenty-two more days. During this time, temperature and pulse remained elevated, scattered areas of bronchial breathing and râles appeared throughout both chests, tubercle bacilli became numerous in the sputum, and she was finally transferred to a hospital for tuberculosis.

In this patient, a pneumococcus pneumonia apparently caused an acute flare-up of a chronic pulmonary tuberculosis. An arterial (anoxic) anoxemia was present with a normal venous saturation. Inhalation of oxygen resulted in slowing of the pulse and in a slight diminution of the cyanosis.

CASE 20 (Table 3)—W. W., white, male, shipping clerk, 20 years. Admitted Jan. 26, 1921.

Diagnosis—Lobar pneumonia.

History—Five days before entry patient was seized with a sudden chill, followed the next day by cough, pain in right chest, fever, and diarrhea. For the last three days he has been mentally confused.

Physical Examination—Jan. 26, 1921 (fifth day of disease). Temperature, 105 F, pulse, 140, respiration, 35. Moderate dyspnea. Slight bluish tinge to finger-nails. Respiration was without expiratory grunt, not particularly shallow or rapid. Slight prostration. Dulness, bronchovesicular breathing, and crepitant rales at right upper lobe. Rest of chest clear. Heart without enlargement, irregularity or murmurs. Pulse of good volume and tension. Urine, sp. gr., from 1.009 to 1.024, occasional slight trace of albumin. White blood cells, 11,000, polymorphonuclears, 83 per cent, three days later 25,100. Blood Wassermann, negative. Blood culture, pneumococcus type I. Sputum, pneumococcus type I.

Course and Treatment—Routine pneumonia treatment. No serum therapy. No digitalis.

January 28 (seventh day of disease). Temperature 104 F, pulse 130, respiration, 45. Condition seemed unchanged. Arterial saturation, 92.5 per cent, venous saturation, 64.1 per cent, arterial carbon dioxide content 36.6 volume per cent, venous carbon dioxide content, 41.4 volume per cent. Carbon dioxide dissociation curve showed a distinct acidosis. No oxygen given.

January 29 (eighth day of disease). Temperature, 103.8 F, pulse, 130, respiration, 40. Patient more distressed. Breathing was more shallow and dyspneic. Cyanosis of nails seemed unchanged. Oxygen was given through the mouthpiece for twenty minutes, patient breathing comfortably and using from 60 to 70 per cent oxygen. Respiration became almost one-half as rapid and much deeper, color appeared to be unchanged. Respiration before, 42, after, 26, five minutes later, 40. Pulse before, from 120 to 124, after, 120, five minutes later, from 116 to 120. Subjectively he noticed no change. Arterial saturation two and one-half hours later, 89.1 per cent, arterial carbon dioxide content, 41.3 volume per cent. That night the patient had a crisis.

February 15. Temperature, pulse and respiration normal. Patient well. Arterial saturation, 96.2 per cent. Carbon dioxide dissociation curve normal.

The single administration of oxygen for twenty minutes had no noticeable effect on the cyanosis or pulse. The breathing was markedly, though temporarily, slowed and deepened. Two and one-half hours later the arterial saturation was slightly less than the day before, indicating no lasting increase in the arterial oxygen and, together with

the lack of improvement in the cyanosis, suggesting that oxygen in this case did not further saturate the hemoglobin. Any effect it had was due to the temporary increase of the oxygen in physical solution in the blood.

CASE 21 (Table 3)—L. S., white, male, laborer, 50 years

Diagnosis—Lobar pneumonia, empyema

History—Twelve days before entry patient developed a chill, pain in right chest, cough and sputum. Fever and prostration continued unabated up to time of admission.

Physical Examination—March 10, 1921 (twelfth day of disease). Temperature, 104 F, pulse, 147, respiration, 50. Marked prostration and dyspnea. Respirations rapid and shallow, accompanied by expiratory grunt. Slight cyanosis of nail beds. Bronchial breathing and dulness over left lower lobe posteriorly, without râles. Heart without enlargement, irregularity, or murmurs. Pulse rapid, small volume. Urine, sp gr, from 1.015 to 1.018 on ten examinations, occasional slight trace of albumin, frequent casts. Blood Wassermann, strongly positive. Blood culture, sterile. White blood cells 26,400, polymorphonuclears, 96 per cent. Sputum, pneumococcus type I.

Course and Treatment—Routine pneumonia treatment. No serum therapy. Digitalis leaves, 1½ grains twice a day from March 12 to March 23.

March 11 (thirteenth day of disease). Temperature, 103 F, pulse 110, respiration, 50. Condition seemed unchanged. Arterial saturation 81.1 per cent, arterial carbon dioxide content, 45.6 volume per cent. Carbon dioxide dissociation curve showed an uncompensated carbon dioxide acidosis.

March 12 (fourteenth day of disease). Temperature, 103 F, pulse, 104, respiration, 46. Pulse slower, otherwise unchanged. Oxygen given through the mouthpiece for thirty minutes, patient breathing about 50 per cent oxygen. Cyanosis of nails decreased but did not disappear. Pulse before, from 100 to 104, after, from 96 to 100. Respirations, from 46 to 48 before, from 46 to 48 after. No subjective changes. Temperature fell to normal that night.

March 14. Temperature, 99 F, pulse, 90, respiration, 28. Slight cyanosis persisted. Arterial saturation, 88.5 per cent, arterial carbon dioxide content, 48.1 volume per cent. Carbon dioxide dissociation curve showed a slight carbon dioxide acidosis. Three days later, temperature again rose and patient was subsequently operated on for empyema. He made a gradual recovery.

In this case, a single administration of oxygen was given. The cyanosis was decreased during the inhalation but did not wholly disappear. The pulse was at times slightly slowed during the administration. No other clinical effects were noted. The arterial anoxemia persisted in part even after the crisis.

DISCUSSION

There were ten patients with lobar pneumonia and two patients with bronchopneumonia treated by oxygen inhalation. All had an arterial anoxemia at some stage of the disease, except one of the cases of bronchopneumonia. (These cases were usually selected because of the presence of cyanosis, and do not therefore indicate the incidence of anoxemia in lobar pneumonia.) Of the ten patients with lobar pneumonia, there were eight in whom blood determinations were done in relation to oxygen therapy. The arterial saturation was increased

in all except one. In four it was raised to the normal level. In the remaining three, all of whom were very anoxic and two of whom had pulmonary edema, the arterial saturation was notably increased by oxygen but did not reach normal limits. In the two cases of lobar pneumonia where the blood determinations did not have a definite relation to oxygen therapy, the cyanosis was decreased but did not seem entirely removed. In the two cases of bronchopneumonia, one showed a normal saturation of the arterial blood before the inhalation but showed a slight fall twenty-four hours later. The second, tested before and directly after the inhalation, showed an increase in arterial saturation. Thus, in the majority of the patients who suffered from anoxemia, it was possible to increase markedly the oxygen of the arterial blood. (Table 4 shows the degree of improvement.) In four of the ten, it was possible to raise the arterial saturation to the normal level. These facts have a distinct bearing on the cause and prognosis of cyanosis in pneumonia.

TABLE 4.—DEGREE OF RELIEF OF ANOXEMIA FOLLOWING OXYGEN THERAPY

Complete Relief				Partial Relief				No Relief			
Case No.	Arterial Saturation		Outcome	Case No.	Arterial Saturation		Outcome	Case No.	Arterial Saturation		Outcome
	Before Oxygen, %	After Oxygen, %			Before Oxygen, %	After Oxygen, %			Before Oxygen, %	After Oxygen, %	
11	85.5	100.0	R	14	74.4	91.6	D	20	92.5	89.1	R
13	94.3	98.0	R	10	62.3	82.2	R				
18	76.8	100.0	R	21	81.1	*	R				
11	90.8	99.7	R	19	87.5	*	R				
				12	*	88.9	R				

* In these three cases, partial relief of anoxemia was shown by diminished cyanosis after oxygen.

R = recovered D = died

It may first be said that cyanosis in pneumonia generally runs parallel to the degree of arterial unsaturation, as was pointed out by Stadie,⁴ (That cyanosis of the nail beds is more frequent in pneumonia and lip cyanosis in cardiac disease was observed by Stadie, and has likewise been our experience. It seems worthy of emphasis that the patients who have a dark grayish or dusky gray color to the nails, in which the blue color is very slight, usually have a severe arterial anoxemia.) Lundsgaard⁵ believes that the degree of cyanosis is dependent on the actual amount of reduced hemoglobin present in the capillary blood. Thus patients who have a high hemoglobin will exhibit more cyanosis than patients with a low hemoglobin, even though the saturation of arterial and venous blood be the same. Slight

⁴ Stadie, W. C. The Oxygen of the Arterial and Venous Blood in Pneumonia and Its Relation to Cyanosis, *J. Exper. M.* **30** 215, 1919.

⁵ Lundsgaard, C. Studies on Cyanosis, *J. Exper. M.* **30** 259, 1919.

cyanosis in cases of anemia becomes of graver significance than in the normal or plethoric individual. For the purpose of directly correlating the degree of cyanosis with the degree of anoxemia, the oxygen unsaturation in c.c. of oxygen per 100 c.c. of blood appears to be the best expression, since this gives the quantity of reduced hemoglobin. For the purpose, however, of estimating the severity of the disease, the oxygen saturation of the blood in per cent is the best expression to use, for this in the arterial blood gives the diffusion pressure at which oxidation in the body takes place. As Barcroft⁶ has shown, the organism is handicapped more by a lowering of the arterial saturation than by a corresponding diminution in oxygen content. In the venous blood, the saturation in per cent gives an indication of blood flow that is not dependent on individual variations in hemoglobin, as are the oxygen unsaturation in volumes per cent or oxygen consumption in volumes per cent.

In Stadie's cases of pneumonia, most of which were influenzal bronchopneumonia but which included seven cases of lobar pneumonia, the arterial oxygen saturation was considered a valuable prognostic sign, the cases with a markedly lowered arterial saturation being usually fatal. In this series, it was also evident that patients with a marked arterial anoxemia were the most desperately ill. A single determination is not, of course, of final significance, as the development of edema or an extension of consolidation alters the arterial saturation just as it changes the other features of the disease. In three of four extremely ill patients, all of whom had a severe arterial anoxemia and all of whom were treated with oxygen, three recovered. The clinical improvement took place as the oxygen in the arterial blood was elevated. In three untreated fatal cases, a marked arterial anoxemia was present in two. It is thus evident that a severe arterial anoxemia, if untreated, is generally followed by a fatal outcome. If the arterial anoxemia is effectively treated, the results in our cases would indicate a distinctly better prognosis.

Concerning the actual cause of cyanosis in pneumonia there is little agreement. Hoover,⁷ stating that all cases of lobar pneumonia have a certain amount of cyanosis which he found proportionate to the area of lung involved, believed it due to an unrespired blood flow through the consolidated lung. According to Haldane,⁸ in areas where complete con-

6 Barcroft, J. Anoxemia, *Lancet* **1** 487, 1920.

7 Hoover, C. F. Oxygen Therapy, *J. A. M. A.* **71** 880 (Sept. 14) 1918.

8 Haldane, J. S. Recent Developments in the Therapeutical Use of Oxygen. Contributions to Medical and Biological Research Dedicated to Sir William Osler **1** 550, 1919.

consolidation occurs the blood supply is commonly diverted Gross⁹ has demonstrated varying degrees of vascular obliteration corresponding to the stages of consolidation It would appear that a certain amount of blood passed through the consolidated lung in red hepatization but practically none in gray hepatization Meakins¹⁰ concluded that the anoxemia of lobar pneumonia was the result of the rapid and shallow type of breathing He found no relation between the degree of anoxemia and the extent of the consolidation¹¹

It is probable that these factors play a somewhat varying rôle If one grants that lobar pneumonia may occur without cyanosis and without arterial anoxemia, and that has been our experience this year, it seems evident that in this type little or no unrespired blood flow passes through the consolidated lung Similarly, in the cases in which oxygen inhalation raises the arterial saturation to the normal, it would appear that the consolidated area had little, if any, blood flow Where the arterial anoxemia is only partially overcome by oxygen inhalation, the persisting cyanosis may be assumed to be due to a certain blood flow through the consolidated area, causing an admixture cyanosis This condition seemed to be a factor in about half of our cases, but it was generally of slight degree, as the major portion of the anoxemia was usually relieved by oxygen therapy In Meakins' two cases of lobar pneumonia reported in 1920,¹² and in two out of the three reported in 1921,¹¹ the arterial saturation was raised to the normal level by oxygen Shallow breathing has only in a few cases been extreme enough to appear to be the chief cause of cyanosis That it is an important factor and that it probably augments the anoxemia is definitely suggested by the work of Haldane, Meakins and Priestley¹³ In the concluding paper of this series, an example of an extreme type of shallow breathing occurring in lethargic encephalitis as the sole cause of a marked arterial anoxemia is described In none of the cases of pneumonia did the shallow breathing approach the extent of these cases of lethargic encephalitis However, the mechanism of its production was clear, and indicates the probability of its occurrence in pneumonia in milder forms There remains for explanation the major cause of the cyanosis

9 Gross Reconstruction of the Circulation of the Liver, Placenta, and Lung in Health and Disease, *Canad M A J* 9 632, 1919

10 Meakins, J C Harmful Effects of Shallow Breathing, with Especial Reference to Pneumonia, *Arch Int Med* 25 1 (Jan) 1920

11 Meakins, J C Observations on the Gases in Human Arterial Blood in Certain Pathological Pulmonary Conditions, and Their Treatment with Oxygen, *J Path & Bacteriol* 24 87, 1921

12 Meakins, J C Therapeutic Value of Oxygen in Pulmonary Lesions, *Brit M J* 1 324, 1920

13 Haldane, J S, Meakins, J C and Priestley, J G The Effects of Shallow Breathing, *J Physiol* 52 433, 1919

From our results, the arterial anoxemia in pneumonia would appear to be largely related to the amount of local or widespread pulmonary edema. Swelling or edema of the alveolar walls and free fluid in the air spaces hinder gas exchange. This condition may occur adjacent to the hepatized area, in other parts of the lung, or widespread through both lungs. When generalized pulmonary edema is present, the hindrance to the mechanical passage of air is present in addition. The increased diffusion pressure of oxygen is able to overcome these resistances to the pulmonary exchange, as was demonstrated in the pulmonary complications of cardiac insufficiency. The fact that the arterial anoxemia is in large part overcome by oxygen administration supports this conception.

Hoover⁷ has himself emphasized the importance of moisture in the lungs as a cause of cyanosis, although he did not believe it to be the usual cause in pneumonia. In edema of the lungs from gas poisoning or cardiac failure, or where moisture was present apart from the consolidation in pneumonia, oxygen therapy relieved the cyanosis but not the air-hunger. This he attributed to an increased carbon dioxide content of the aortic blood that resulted from the disturbance in respiratory exchange caused by foam or edema in the bronchial tree. This theory of carbon dioxide retention has recently received confirming evidence from Means and his collaborators¹⁴ in pneumonia and Peters and Barr¹⁵ in cardiac decompensation. Carbon dioxide dissociation curves have shown that in both these conditions there may be a carbon dioxide retention in the arterial blood of the extent to cause an actual change in p_H .

Concerning the dyspnea of lobar pneumonia, it may be said that anoxemia plays but a limited rôle. There was no constant relief of respiratory distress after oxygen therapy. Breathlessness would seem to be more closely related to a faulty pulmonary ventilation that was insufficient to get rid of carbon dioxide rather than insufficient to acquire oxygen. The alkali reserve was within normal limits, except in a few cases where it was only slightly lowered. The carbon dioxide content of arterial and venous blood was usually a little increased by oxygen therapy, but at times unaffected. These relationships will be discussed fully in a paper in which carbon dioxide dissociation curves in pneumonia are presented. In several tested cases, the blood bicarbonate

14 Means, J. H., Bock, A. V., and Woodwell, M. W. Studies of Acid-base equilibrium in Disease from the Point of View of Blood Gases, *J. Exper. Med.* **33**:201, 1921. Barach, A. L., Woodwell, M. N., and Means, J. H. The Reaction and Available Blood Alkali in Pneumonia. To be published.

15 Peters, J. P., and Barr, D. P. The Carbon Dioxide Absorption Curve and the Carbon Dioxide Tension of the Blood in Cardiac Dyspnea, *J. Biol. Chem.* **45**: 559, 1921.

showed a definite elevation as convalescence was reached. This fact and its bearing on the administration of alkali will be discussed in another paper¹⁶

A fact brought out in connection with the inhalation of oxygen is the increase in the amount of circulating hemoglobin under varying conditions of anoxemia and its decrease after relief of the anoxemia. This relationship was more constant in the cardiac than in the pneumonia cases, although exceptions were common to both groups. The effect of oxygen was in increasing the number of red blood cells and hemoglobin in peripheral blood has long been known and is familiarly manifested in patients with congenital heart disease and in people who live at high altitudes. In the Pike's Peak expedition, Miss Fitzgerald¹⁷ found that the increase in hemoglobin was proportionate to the increase in altitude, that is to say, the anoxemia. In the patients with chronic gas poisoning suffering from anoxemia, Barcroft¹⁸ found a noteworthy fall in the red blood cells and hemoglobin after they had lived in an oxygen chamber five days. An insufficient oxygen supply has been shown to cause a definite increase in hemoglobin in one hour¹⁹. In one of our cases (Case 10), a fall in arterial saturation from 75 to 62 per cent in one day resulted in an increase in oxygen capacity (representing the hemoglobin) from 20.7 to 22.3 volume per cent. On the following day, the inhalation of oxygen raised the arterial saturation to 82 per cent, and the oxygen capacity dropped to 20.7 volume per cent again. In another case (Case 8), the inhalation of oxygen raised the arterial saturation to the normal in two hours and was accompanied by a decrease in capacity from 21.5 to 20.6 volume per cent.

This effect of oxygen was on the amount of circulating hemoglobin is emphasized particularly because the variations in the oxygen capacities of Stadie's cases⁴ of pneumonia has been made the reason for criticism and for an assumption by Meakins¹¹ of inaccuracies in the Van Slyke methods. When Stadie's cases are examined, it is found that the majority of them show this fundamental mechanism. In his most marked case (Case 35) a severe arterial anoxemia gradually diminished as the patient recovered, and the oxygen capacity correspondingly diminished from 19.1 to 14.9 volume per cent. The

16 Means J. H. and Barach, A. L. Remarks on the Symptomatic Treatment of Pneumonia. Read at the Association of American Physicians, May 10, 1921.

17 Fitzgerald. Quoted from Haldane, J. S., *Organism and Environment* 1917, p. 52.

18 Barcroft J., Hunt, G. H., Dufton D. The Treatment of Chronic Cases of Gas Poisoning by Oxygen Administration in Chambers, *Quart. J. M.* **13** 179 1920.

19 Gregg, H. W., Lutz B. R., Schneider, E. C. The Changes in the Content of Hemoglobin and Erythrocytes of the Blood in Man During Short Exposures to Low Oxygen. *Am. J. Physiol.* **1** 216, 1919.

average oxygen capacity of his nonfatal cases, characterized by little cyanosis, was 20 volume per cent, whereas the average of the fatal cases who uniformly had a marked cyanosis was 23 volume per cent. The nonfatal cases showed a decrease in oxygen capacity as they recovered and as they became free from oxygen want. In the cases of cardiac decompensation of Lundsgaard²⁰ this tendency can also be observed. A striking case (Case 3) showed a fall in capacity from 22.5 to 17.5 volume per cent as compensation was regained and cyanosis disappeared. Cunningham²¹ observed a patient with edema of the lungs at the height of the attack and when he was normal, and the oxygen capacities were, respectively, 25 and 19 volume per cent. Underhill and Ringer²² studied cases of influenzal bronchopneumonia and found variations in hemoglobin from day to day, as high as from 20 to 30 per cent. Here, also, the hemoglobin increased as the patients became worse and decreased as they improved. This general relationship of an increase in hemoglobin to oxygen want is by no means constant in clinical disease, but it must be remembered that there are a multiplicity of factors that govern the amount of hemoglobin circulating in the blood. Schneider and Havens²³ found an increase in hemoglobin due to physical exertion, abdominal massage and pressure, injection of adrenalin, and during athletic training. Changes in the blood volume result inevitably in changes in hemoglobin, and Buckman²⁴ has demonstrated that such changes occur in pneumonia, the plasma volume being decreased during the course of the disease and returning to normal during convalescence. Variations in hemoglobin corresponding to the different periods of the day have been demonstrated, and, according to Dreyer, Bazett and Pierce,²⁵ may reach as high as 30 per cent while 10 per cent changes are of more or less common occurrence. In consideration of these facts, it would appear that the occurrence in pneumonia of a changing oxygen capacity offers no basis for the assumption of inaccuracies in method but seems rather to demonstrate a fundamental physiological mechanism.

The venous saturation was determined in ten cases of lobar pneumonia and two cases of bronchopneumonia. A true stagnant anoxemia

20 Lundsgaard, C. Studies of Oxygen in the Venous Blood. Determinations on Five Patients with Uncompensated Circulatory Disturbances. *J Exper M* **27** 228, 1918.

21 Cunningham, D. Quoted from Bock, A. V., Constancy of the Plasma Volume in Disease, *Arch Int Med* **27** 83 (Jan) 1921.

22 Underhill, F. P., and Ringer, M. Blood Concentration Changes in Influenza with Suggestions for Treatment, *J A M A* **75** 1531 (Dec 4) 1920.

23 Schneider, E. C., and Havens, L. C. Changes in the Blood After Muscular Exertion and During Training, *Am J Physiol* **36** 239, 1915.

24 Buckman, Thomas E. Personal communication. To be published.

25 Dreyer, G., Bazett, H. C., Pierce, H. F. Diurnal Variations in the Hemoglobin Content of the Blood, *Lancet* **1** 588, 1920.

was shown in one case of lobar pneumonia, in four others a relative lowering of the venous saturation occurred, due to the diminution of the arterial saturation. One of the cases of bronchopneumonia had a true stagnant anoxemia a short time before death. In influenzal bronchopneumonia Harrop²⁶ found the venous unsaturation normal up to the time of death. As discussed in the previous paper, a venous saturation below 65 per cent does not indicate an impaired circulation unless the arterial saturation is normal. The normal difference between arterial and venous saturation, or coefficient of utilization, being between 20 and 30 per cent, and the results in this series lying for the most part within this range, would indicate a normal or increased blood flow in uncomplicated pneumonia.

In seven of the above cases, the venous blood was tested in relation to oxygen therapy. The venous saturation was increased in four, in three of which the rise was somewhat greater than could be accounted for by the arterial elevation, suggesting a temporarily increased blood flow. One of the most constant objective changes due to oxygen therapy was the slowing of the pulse, which apparently allowed the blood flow to be maintained at less effort from the heart.

Attention might be called to the similarity between the symptoms and signs of experimental anoxemia and anoxemia as it occurs in clinical disease. The elevated pulse, irrational states and delirium, cyanosis, rapid shallow or periodic breathing are common to patients with pneumonia or heart disease, as they are to individuals who have been experimentally deprived of oxygen. These harmful effects of anoxemia are, in the main, capable of relief or prevention. The patient is thus in better condition to withstand the other factors in the disease. When acute oxygen want supervenes, as in the development of pulmonary edema, a fatal outcome may be averted by overcoming the anoxemia. The patient is then in no sense cured, but he is given a renewed opportunity to cope with the remainder of the disease. If he can be tided over to the period of the crisis he has the chance of a swift decline in all his symptoms that would otherwise be lost to him. The effect of a single inhalation of oxygen in raising the arterial saturation is in the main temporary, although there is evidence that in cases in which shallow breathing is pronounced it may be more permanently elevated. Prolonged administration or frequently repeated administration can, however, keep the patient free from anoxemia most of the time, and has been attended by a diminution in the signs of lung edema. As Haldane²⁷ has observed "It may be argued that such measures as the administration of oxygen are

26 Harrop, G. A. Behavior of the Blood Towards Oxygen in Influenzal Infections, *Bull. Johns Hopkins Hosp.* 30 10, 1919.

27 Haldane, J. S. The Therapeutic Administration of Oxygen, *Brit. M. J.* 1 182, 1917.

at the best only palliative and of no real use, since they do not remove the cause of the pathological condition. As a physiologist, I cannot for a moment agree with this reasoning. The living body is no machine, but an organism constantly tending to maintain or revert to the normal, and the respite offered by such measures as the temporary administration of oxygen is not wasted but utilized for recuperation."

To combat anoxemia, oxygen must be given effectively. The Haldane apparatus is effective and convenient, if the patient can be made to bear the mask. The simple mouthpiece rebreathing method we have found effective and productive of minimal discomfort to the patient. The oxygen bed tent described by Leonard Hill²⁸ would appear to be very efficient and certainly merits further trial.

SUMMARY

1 There have been observed in all eleven patients with lobar pneumonia, each of whom had an arterial anoxemia at some stage of the disease, and four patients with bronchopneumonia, two of whom had an arterial anoxemia.

2 Ten patients with lobar pneumonia were treated by the inhalation of oxygen. In eight, the blood gas determinations were done before and after oxygen therapy. Of these, the arterial oxygen saturation was increased in all except one. In four, it was raised to the normal level.

3 Two patients with bronchopneumonia who had no arterial anoxemia were treated with oxygen. In one, the arterial saturation was shown to be increased directly after the inhalation. In the second, analysis twenty-four hours later showed a slight fall in arterial saturation.

4 A true stagnant anoxemia was demonstrated in one of ten cases of lobar pneumonia. In four other cases there was a relative lowering of the venous saturation due to the diminution of arterial oxygen. The difference between the arterial and venous saturation was generally normal or less than normal, indicating that a normal or increased blood flow is usually present in uncomplicated pneumonia.

5 The most consistent changes in the clinical condition of the patient were the clearing of the cyanosis and slowing of the pulse. The respiratory rate was sometimes slowed, the mental condition of the patient was frequently improved, the dyspnea was not usually relieved.

²⁸ Hill, L. A Simple Oxygen Bed Tent and Its Use to a Case of Oedema and Chronic Ulcer of the Leg, *J. Physiol.* **50** (May 24) 1921, *Proc. Physiol. Soc.*, p. 20.

6 Oxygen inhalation for one-half hour was sufficient in the mild or moderate cases of anoxemia to elevate the arterial saturation and cause clinical improvement. In the severe cases, one to two hours was necessary. The effect of a single administration was, in the main, temporary. The effect of repeated and prolonged administration produced persistent beneficial changes in the oxygen saturation of the blood, the pulse, breathing, color, comfort, and mental condition of the patient.

7 In three patients in whom a condition of acute oxygen want followed the development of pulmonary edema, the prolonged administration of oxygen resulted in striking clinical improvement, and seemed to avert a fatal outcome.

8 It is believed that oxygen therapy has a rational rôle in the treatment of pneumonia.

9 A convenient effective method of giving oxygen has been developed which does not cause discomfort to the patient.²⁹

²⁹ The method of administering oxygen is described in Paper I of this series. The apparatus, with a few recent additions, can be secured from Mr. Warren E. Collins, 584 Huntington Avenue, Boston.

STUDIES IN OXYGEN THERAPY

III IN AN EXTREME TYPE OF SHALLOW BREATHING OCCURRING IN LETHARGIC ENCEPHALITIS ^

ALVAN L. BARACH, M.D.

AND

MARGARET N. WOODWELL, A.B.

BOSTON

The effects of oxygen inhalation on types of anoxemia frequently present in clinical disease, as those associated with cardiac insufficiency and pneumonia, have been described in Papers I and II of this series. The terminal development in two cases of lethargic encephalitis of a rare type of shallow breathing resulted in sudden extreme arterial anoxemia and carbon dioxide retention in arterial and venous blood. The effects of oxygen inhalation on the blood gases and on the clinical signs are reported in this paper.

REPORT OF CASES

CASE 25 (Table 2) —M. S., white, male, auto repairer, age, 25 years

Diagnosis—Lethargic encephalitis

History—Two weeks before entry, patient had right temporal headache. This was followed by pains in both arms and across the abdomen. During the three days prior to admission he developed fever, insomnia, blurring of vision and drooping of left eyelid.

Physical Examination—Jan. 8, 1921. Temperature, 101 F., pulse, 115, respiration, 25. Slight ptosis of both eyes, diplopia, weakness of left internal rectus muscle. Lungs clear and resonant. Heart not enlarged, no murmurs. Reflexes not abnormal. Urine negative. White blood cells, 11,100, polymorphonuclears, 79 per cent. Lumbar puncture revealed clear fluid under increased pressure, cells, 44, polymorphonuclears 14 and mononuclears 30, alcohol positive, ammonium sulphate negative, Wassermann negative.

Course and Treatment—Jan. 10, 1921. At 1 a. m. he became suddenly dyspneic and cyanotic, temperature rose to 103 F., pulse to 140, respiration to 48. At 10 a. m. he was comatose, deep and superficial reflexes could not be obtained, neck was stiff, the extremities were limp and flaccid. No Kernig was present, no Babinski or ankle clonus. Lungs clear and resonant throughout. He breathed in a rapid jerky manner, at each inspiration the upper abdomen underwent a double convulsive contraction with a simultaneous twitching of the neck muscles but without any movement of the intercostal muscles during either phase of respiration. His face was of a dusky grayish color, the lips deeply cyanotic, the nail-beds a light blue color. Oxygen had been administered during the night through a funnel held close to the nose, without alleviation of the cyanosis in any degree. The mask of the rebreathing apparatus was applied with a valve adjustment, giving about an 80 per cent oxygen.

* From the Medical Service of the Massachusetts General Hospital. This paper is No. 17 of a series of studies of the physiology and pathology of the blood from the Harvard Medical School and allied hospitals.

* The expense of this work was, in part, defrayed by a grant from the Proctor Fund of Harvard University for the study of chronic diseases.

mixture It was then noticed that the rebreathing bag barely moved, and that an extreme type of shallow breathing was the cause of the cyanosis This was in marked contrast to the convulsive movements of the upper abdomen, which suggested moderately deep breathing At the end of five minutes a slight diminution of the cyanosis became noticeable, at the end of ten minutes it was strikingly diminished, after twenty-five minutes there was no cyanosis of face, fingers or mucous membranes, the nails were pink, the ears a rosy red, the lips free from any blueness At the same time, the respiratory rate decreased from 45 to 34 and the pulse from 141 to 105 and later to 98 The extreme shallow character of the respiration persisted unchanged, but the spasmodic movements of the diaphragm had largely disappeared The neck was no longer stiff All the reflexes persisted absent

TABLE 1 — EFFECT OF OXYGEN INHALATION ON PULSE, RESPIRATION AND CYANOSIS IN EXTREME SHALLOW BREATHING IN CASE 25

Time	Pulse	Respiration	Cyanosis
10 25 Before oxygen	141	45	++++
10 35 During oxygen	132		++
10 45 During oxygen	120		+
10 55 During oxygen	105	38	0
11 00 During oxygen	98		0
11 20 During oxygen	112	40	0
11 28 Oxygen stopped for 3 min	126		++
11 33 During oxygen	114	34	+
11 38 During oxygen	114		0
11 43 Oxygen stopped for 5 min	141		++++
11 55 During oxygen	132	44	++
12 02 p m During oxygen	126	40	+
12 09 During oxygen	123		+
12 26 During oxygen	124	40	+
12 34 During oxygen	129	42	++
12 53 During oxygen	126		++
12 56 During oxygen	126		++
1 00 During oxygen	140	39	++
1 05 During oxygen	129		++
1 10 During oxygen		39	++
1 16 During oxygen	120		++
1 23 During oxygen	124	40	++
1 35 During oxygen	128		++
1 45 During oxygen	140	40	+++
2 00 During oxygen	140		+++
2 20 During oxygen	140	40	+++

The inhalation of oxygen relieved the arterial anoxemia and resulted in a striking slowing of the pulse The withdrawal of oxygen caused a simultaneous increase in the anoxemia and in the pulse rate The arterial oxygen saturation was raised from 60.7 to 84.5 per cent in forty-five minutes The arterial carbon dioxide content in that time mounted from 64.2 volume per cent to 88.4 volume per cent, indicating a steadily increasing carbonic acidosis Failure of the circulation finally occurred, and accounted for the reappearance of the cyanosis

The oxygen saturation of the arterial blood before the inhalation was 60.7 per cent, the venous saturation was 46.4 per cent, the arterial carbon dioxide content, 64.2 volume per cent, the venous carbon dioxide content, 68.4 volume per cent After oxygen had been given for forty-five minutes, the arterial saturation was 84.5 per cent, the arterial carbon dioxide content, 88.4 volume per cent, after fifty-five minutes the venous saturation was 87.9 per cent, the venous carbon dioxide content, 88.6 volume per cent When oxygen had been given for one hour it was stopped for three minutes The cyanosis returned and the pulse began to climb, reaching 126 Oxygen was recommenced and given for five minutes, when the tank gave out Pulse was then 114 It was five minutes before another tank had been secured, and in the meantime pulse rose to 141, respiration to 44, color changed to deep cyanosis of lips and finger nails Oxygen was begun again but with less relief than formerly Pulse slowed to 126 after the first hour and then gradually became elevated and feeble Cyanosis increased moderately as circulation began to fail After three more hours oxygen was stopped and patient died three minutes later in deep cyanosis (See Table 1 for effect on pulse and respiratory rates)

CASE 26 (Table 2)—E D, white, housewife, age, 23 years

Diagnosis—Lethargic encephalitis

History—Ten days before entry an aching pain appeared behind right ear. Headache persisted up to time of admission, confined to right temporal region and variable in severity. She developed pains in chin, neck and shoulder blades. When the pains in the shoulders occurred respiration became very rapid and jerky (husband's statement). For seven days blurred vision and diplopia were present. Felt sleepy but found it difficult to go to sleep.

Physical Examination—Jan 23, 1921. Temperature, 101 F, pulse, 130, respiration, 40. Double ptosis, double facial paralysis. Fields of vision limited in both eyes. Right leg twitches involuntarily. Knee-jerks not obtained. No other abnormal reflexes. Heart and lungs not abnormal. Urine negative. White blood cells, 11,200, polymorphonuclears, 75 per cent. Blood Wassermann, negative. Nonprotein nitrogen 53.1 mg per 100 cc. Spinal fluid under increased pressure, 20 cells, 14 polymorphonuclears and 6 lymphocytes. Alcohol negative. Ammonium sulphate negative. Culture sterile. Wassermann negative.

TABLE 2—EFFECT OF OXYGEN INHALATION ON THE BLOOD GASES IN EXTREME SHALLOW RESPIRATION OCCURRING IN TWO CASES OF LETHARGIC ENCEPHALITIS

Case No	Diagnosis	Date, 1921	Oxygen Inhalation	Oxygen Content		Oxygen Saturation		Oxygen Capacity Vol %	Hemoglobin Calculated from Oxygen Capacity	Carbon Dioxide Content	
				Arterial Vol %	Venous Vol %	Arterial %	Venous %			Arterial Vol %	Venous Vol %
25	Lethargic encephalitis	1/10	Before oxygen	14.74	11.29	60.7	46.4	24.30	131.2	64.2	68.4*
			Oxygen for 1 hr 30 min	20.29		84.5		(24.30)	(131.2)	88.4†	
			Oxygen for 1 hr 40 min		21.37		87.9	(24.30)			88.6†
26	Lethargic encephalitis	1/25	Before oxygen	13.76		64.5		21.31	115.0	51.0	
			Oxygen for 45 min	17.44		82.0		(21.31)	(115.0)	62.0	

The oxygen saturation of the arterial and venous blood was raised. The carbon dioxide content of the blood steadily increased.

* Carbon dioxide dissociation curve showed an acidosis.

† This value is approximate because the combined gases were above the calibrated portion of the pipet.

Course and Treatment—Jan 25, 1921. Temperature, 102.5 F, pulse, 140, respiration, 45. At 7:30 a.m., patient went into coma, became cyanotic and breathed in a rapid, jerky manner. Lips and ears showed moderate cyanosis, the finger nails very marked cyanosis. Moist râles present at right base. Pulse of poor quality, rate 141. Oxygen was given through a mask with valve attachment providing about an 80 per cent oxygen mixture for three hours. The first effect noticeable was a definite slowing of the pulse. In three minutes it slowed from 141 to 132, in fifteen minutes it had attained its maximal slowing, 114, after this the pulse rose and showed only slight or temporary periods of slowing. The cyanosis began to diminish in five minutes and became progressively less for twenty minutes, at that time there was just a slight bluish tinge to the nail beds which never wholly disappeared. Respirations continued very shallow and rapid, rate from 45 to 50. The cyanosis remained minimal for the first hour, increased in the second hour and was marked in the third hour. Whenever the oxygen was removed she became darkly cyanotic. (The persistence of the cyanosis in the presence of a rich oxygen mixture seemed due to the exceptional shallowness of the respiration and the stagnant circulation.) After three hours' oxygen the inhalation was

stopped and the patient died two minutes later Ten minutes previous, 25 gm sodium bicarbonate were given intravenously without effect

The arterial oxygen saturation before the inhalation was 64.5 per cent, after forty-five minutes, 82 per cent Arterial carbon dioxide content before was 51 volume per cent, after, 62 volume per cent The carbon dioxide dissociation curve before oxygen showed a marked uncompensated carbon dioxide acidosis, *pH* 7.22

In both these cases the soda-lime was tested before and after using and found perfect for absorption of carbon dioxide

In these two cases shallow breathing was the direct cause of a very marked arterial anoxemia Because of the convulsive movements of the diaphragm the extreme shallowness of the respiration was not actually appreciated until the mask was applied, when it was noticed that the rebreathing bag barely moved Interpretation of this unique phenomenon in lethargic encephalitis cannot be made with certainty as necropsies were not obtained It seems probable, however, that the respiratory center in the medulla became involved in the inflammatory process, since lesions in the bulb are quite common in this disease

Shallow breathing has been shown to be a cause of anoxemia by producing uneven ventilation of the lungs, and consequently incomplete oxygenation of the blood¹ This is based on the conception of Keith² that expansion of the lungs takes place in a manner resembling the opening of a Japanese fan, the peripheral portions expanding first and the root region last In these cases the total ventilation itself was so small that it was the air in the trachea and bronchi that became chiefly renewed and the alveolar air only secondarily changed

The effects on the blood gases were first, a marked arterial anoxemia, and secondly, a retention of carbon dioxide in the arterial and venous blood Oxygen therapy was able to increase greatly the arterial and venous oxygen saturation, but was powerless against the steady accumulation of carbon dioxide In one case a carbon dioxide dissociation curve, done before oxygen was given, showed an uncompensated carbon dioxide acidosis, *pH* 7.22

In the beginning, the circulation was strikingly improved as a result of the relief of the anoxemia (Table 1 shows the parallel effect on the pulse and cyanosis of the inhalation of oxygen) The subsequent failure of the circulation seemed dependent on the increasing carbon dioxide retention in the blood, which showed no disposition to decrease since the shallowness of respiration itself persisted The final cyanosis was thus of stagnant origin

1 Haldane, J S Meakins, J C, and Priestley, J G The Effects of Shallow Breathing, *J Physiol* **52** 433, 1919

2 Keith, A Quoted from Haldane, J S, Meakins, J C, and Priestley, J G The Effect of Shallow Breathing, *J Physiol* **52** 449, 1919

SUMMARY

1 In two patients with lethargic encephalitis the development of an extreme type of shallow breathing was attended with deep cyanosis and coma. The arterial blood was markedly deficient in oxygen and contained an excess of carbon dioxide.

2 Inhalation of oxygen greatly relieved the arterial anoxemia but was without effect on the steady accumulation of carbon dioxide. An uncompensated carbon dioxide acidosis, p_H 7.22, was demonstrated in one case by a carbon dioxide dissociation curve.

3 The circulation was strikingly improved in the beginning as a result of the relief of the anoxemia. Later, progressive cardiac failure occurred, apparently related to the carbon dioxide retention.

4 It is evident that shallow respiration, if extreme, interferes not only with oxygen absorption but with carbon dioxide elimination.

5 It seems probable that a terminal involvement of the respiratory center in lethargic encephalitis is at times the cause of death.

THE TOTAL NONPROTEIN NITROGEN CONSTITUENTS OF THE BLOOD IN CHRONIC NEPHRITIS WITH HYPERTENSION⁺

J LISLE WILLIAMS, M D

CHICAGO

During the course of investigations regarding the presence and amount in the blood of various nitrogenous waste products usually excreted by the kidneys in patients with essential arterial hypertension but without nephritis, similar studies were made on eighty-eight patients with chronic nephritis and hypertension or with myocardial decompensation. Although similar studies have been reported by others, it is believed the results presented here will be of interest and afford suitable control to those previously reported ¹

The patients studied are divided into three groups. All had arterial hypertension. Group I includes the patients with uremia, Group II those with a moderate increase in the various nonprotein nitrogen constituents of the blood, and Group III those with a slight increase.

Table 1 includes the maximum, minimum and average values for the urea, total nonprotein nitrogen, uric acid and creatinin of the blood for each of the three groups.

There are thirteen patients in Group I, all with a marked retention of nonprotein nitrogen compounds in the blood and all eventually died of uremia. Albumin was present in the urine of every patient and casts were present in all but two. Before the onset of uremia, there was a progressive increase in the amount of waste nitrogen retained in the blood with the progress of the disease. Nine patients were studied before and after coma supervened. As a rule, with the appearance of coma the various nitrogenous extractives of the blood were present in higher concentration than before. The unconsciousness lasted from two days to two weeks before death, and as it deepened there was an increase in the amount of the nonprotein nitrogen constituents of the blood. With two patients there was a terminal acidosis, as determined by the depression of the alkali reserve.

The ages in this group varied from 27 to 80 and averaged 45.2 years, the maximum systolic blood pressure varied from 176 to 272 mm, the diastolic from 75 to 170 mm, the phenolsulphonaphthalein test of renal function from a trace to 30 per cent, average 10.2 per

*From the Pathological Laboratory, St. Luke's Hospital, aided by the Seymour Coman Fund.

¹ Arch. Int. Med. 27:748 (June) 1921.

cent in two hours. The Wassermann reaction of the blood for syphilis was positive in one patient and negative in nine patients, in three it was not learned.

There was no relationship between the height of the blood pressure and the degree of nitrogen retention in the blood nor was there any constant relationship between any of the symptoms and the amount of the nonprotein nitrogen in the blood. In general, the largest amount of uric acid was found in the blood of those patients with most marked cardiac decompensation or the retention of uric acid was most marked with the development of the decompensation.

Ophthalmoscopic examination of four patients revealed a hemorrhagic retinitis in two, and a chronic nephritis retinitis in two, one of whom had retinal hemorrhages. All thirteen patients of this group died.

The duration of life after blood was taken for chemical examination varied from two days to seven months, the average being fifty-eight days. The time limit in prognosis could not be foretold accurately by chemical examination of the blood, but generally the higher values for

TABLE 1—NONPROTEIN NITROGEN CONSTITUENTS IN ONE MG PER 100 C C OF BLOOD

Group	Urea N			Total N-P-N			Uric Acid			Creatinin		
	Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age
I	259.8	61.8	108.7	287.6	83.8	146.3	22.6	5.4	11.2	29.5	4.2	11.7
II	69.0	30.3	39.3	101.0	45.4	61.8	9.2	4.9	6.2	11.9	1.6	2.9
III	29.9	17.8	22.9	57.3	28.6	43.2	15.6	1.5	5.3	4.0	1.2	2.2

creatinin (average 17.6 mg) were obtained in patients living only a short time (average twenty-six days). One patient, however, lived four months after a blood creatinin of 20.4 mg per 100 c c had been obtained.

Postmortem examination of the bodies of two patients revealed a marked chronic diffuse nephritis with secondarily contracted kidneys, both with marked hypertrophy of the heart and a moderate degree of sclerosis of the aorta and larger arteries. Therefore, from chemical, functional and anatomic evidence coincident with clinical experience these thirteen patients with hypertension had chronic nephritis and died of uremia.

Included in Group II are thirty-six patients with a moderate degree of nitrogen retention in the blood. Their ages varied from 30 to 70, and averaged 52.4 years. The disease in five of these was diagnosed clinically as myocardial degeneration or myocarditis² with cardiac

² This diagnosis of "myocarditis" is an error, since the alterations microscopically are edema and passive hyperemia without cellular exudate.

decompensation Only two patients had neither albumin nor casts in the urine

The systolic blood pressure ranged from 160 to 284 mm, the diastolic from 70 to 180 mm mercury, the phenolsulphonephthalein test from 2 to 60 per cent for the two hour period

The Wassermann test for syphilis on the blood was positive in four patients and negative in twenty-three, but four of these gave a history of syphilis

Dyspnea, headache and nocturia were the most prominent symptoms Headache, nausea and vomiting were the symptoms most commonly associated with higher values for retained nitrogen, but this relationship was not constant Headache, nausea and vomiting were much more common in this group than in Group III where the increase in the various nonprotein nitrogen substances is slight

Ophthalmoscopic examination of twelve patients revealed a marked hemorrhagic retinitis in five, a chronic degenerative retinitis in seven with retinal hemorrhages in three, marked venous engorgement and arteriosclerotic changes in one each The patients having the hemorrhagic neuroretinitis had the highest values for nonprotein nitrogen of the group and the mortality was 60 per cent

Of the thirty-six patients, fourteen were known to be living for from three months to two years after the chemical examinations of the blood were made Eleven of these fourteen patients were improved on leaving the hospital, two were unimproved and one patient was worse Fourteen patients died and the condition of eight could not be learned Of the fourteen patients who died, five died of uremia or of uremia and a contributory cause All five patients with cardiac decompensation died, and postmortem examination of two of these demonstrated a terminal bronchopneumonia Both had a moderate chronic diffuse nephritis with arteriosclerotic atrophy, and both had a marked sclerosis of the aorta and larger arteries, one patient being syphilitic, the other senile Four patients died of cerebral hemorrhage For two, this diagnosis was confirmed by postmortem examination, and both had a moderate chronic diffuse nephritis, one with secondarily contracted kidneys, the other arteriosclerotic atrophy

There are thirty-nine patients in Group III varying from 27 to 72 years and averaging 52 years The clinical diagnosis for twelve was cardiac decompensation, for six cardiorenal disease, three essential arterial hypertension and eighteen chronic nephritis with hypertension

The urine of twenty-seven patients contained both albumin and casts, that of ten albumin alone, that of one, a few hyalin casts, and neither albumin nor casts in one

The maximum systolic blood pressure varied from 150 to 268 mm mercury, the diastolic from 74 to 164 mm. The phenolsulphonephthalein test varied from 20 to 76 per cent and averaged 42 per cent for the two hour period. The Wassermann reaction of the blood for syphilis was negative on twenty-six patients.

Dyspnea was the most common symptom and was present with eighteen patients. Twelve patients complained of headache, eight of cough, seven each of pain or distress in the precordium, and nausea and vomiting, six each of frequency of urination and the results of a paralytic stroke some time before, five each of orthopnea, visual disturbances, palpitation, nocturia, dizziness and weakness, four of constipation and pain or distress in the epigastrium, three a generalized edema, and scanty urination, two each had dysuria, migraine, nervousness, sleeplessness, loss of weight and syncope. With seven patients the hypertension had been present for from five to twenty years (average 8.2 years). Hypertrophy of the heart was the most common abnormality found on physical examination in twenty-six patients. Nineteen patients had edema of the feet and legs, which was marked in eight, in twelve patients the liver was enlarged, in ten each there was the systolic murmur of mitral regurgitation and marked sclerosis of the peripheral arteries, in nine there was pulmonary hypostasis, in seven an ascites, in five the aortic second sound was distinctly accentuated, in four there was a marked scrotal edema, in three there was a hemiplegia, and sluggish and unequal pupils, in two each there was a gallop rhythm, a palpable kidney, bilateral hydrothorax and the murmur of aortic regurgitation.

After a period of from two days to two and one half years, twenty-four patients were known to be living, eleven were dead and regarding four no information is available. Seven of the living were unimproved, fourteen were improved and three were worse. Five of the eleven deceased died of "heart failure" and for two of these post-mortem examination revealed marked hypertrophy and dilatation of the heart, with marked senile sclerosis and arteriosclerotic kidneys in one, cardiac hypertrophy, dilatation and mitral stenosis in the other, four died of cerebral hemorrhage, and for two of these this was confirmed by postmortem examination, and one of uremia but without necropsy.

No relationship was found between the height of the blood pressure and the degree of nitrogen retention in any of the patients in Group III.

The patients with clinical evidence of myocardial insufficiency including dyspnea, cough, palpitation, engorgement of the liver, and edema of the lower extremities had an increased concentration of uric acid in the blood, average 6.73 mg as opposed to 3.86 mg per 100 c.c. for the patients without marked evidence of decompensation. Improvement

of the decompensation was accompanied by a marked diminution in the amount of uric acid in the blood whereas the other nonprotein nitrogen substances of the blood were only moderately decreased in amount

As a rule, the higher values for blood urea, total nonprotein nitrogen, and creatinin were obtained in those patients where the clinical evidence was in favor of chronic nephritis or where a chronic nephritis was found at postmortem examination

DISCUSSION

These results are in accord with the observations of other investigators who have studied the functional pathology of the kidneys in chronic nephritis. It has been known for many years that there is an increased amount of nitrogenous urinary waste in the blood of patients with chronic interstitial nephritis. The amount of urea thus retained in the blood was estimated fairly accurately by Herter³ and was found by him to be increased in chronic nephritis. Concomitant with the rise in the urea nitrogen of the blood is the increased concentration of the total incoagulable nitrogen as has been found by numerous investigators among whom may be mentioned Strauss,⁴ Folin,⁵ Farr and Austin,⁶ Hopkins and Jonas,⁷ and Myers.⁸

It is now generally admitted that chronic parenchymatous or degenerative nephritis is not associated with such marked retention of "noncolloidal" nitrogen in the blood as are the various types of chronic nephritis associated with arterial hypertension. In most patients uremia which occurs as a sequel to chronic interstitial nephritis is accompanied by a high concentration of the nonprotein nitrogen compounds in the blood. Very exceptionally does uremia occur without these coincident phenomena and one such patient (blood pressure, 140-150 mm) has been reported by Foster⁹ who found only twenty-eight mg of non-

3 Herter, C. A. On Urea in Some of Its Physiological and Pathological Relations. *Johns Hopkins Hosp. Rep.* **9** 69, 1900.

4 Strauss, H. Die Chronischen Nieren Entzündungen in ihre Einwirkung auf Blutflussigkeit und deren Behandlung. Berlin, 68 1902.

5 Folin, O., Denis, W. and Seymour, M. The Nonprotein Nitrogen Constituents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism. *Arch. Int. Med.* **13** 224 (Feb.) 1914, Folin, O. and Denis, W. On Uric Acid, Urea, and Total Nonprotein Nitrogen in Human Blood. *J. Biol. Chem.* **14** 29, 1913.

6 Farr, C. B. and Austin, J. H. The Total Nonprotein Nitrogen of the Blood in Nephritis and Allied Conditions. *J. Exper. Med.* **18** 228 1913.

7 Hopkins, A. H. and Jonas, L. Studies in Renal Function with Special Reference to Nonprotein Nitrogen and Sugar Concentration in the Blood. Phthalein Elimination and Blood Pressure. *Arch. Int. Med.* **15** 964 (June) 1915.

8 Myers, V. C. and Fine, M. S. The Nonprotein Nitrogenous Compounds of the Blood in Nephritis with Special Reference to Creatinin and Uric Acid. *J. Biol. Chem.* **20** 391 1915.

9 Foster, N. B. Functional Tests of the Kidney in Uremia. *Arch. Int. Med.* **13** 452 (May) 1913.

protein nitrogen per 100 c c of blood. On postmortem examination there was found a marked chronic nephritis (small granular kidneys), edema of the brain and colitis. It is questionable whether such a disease and its termination should be classified with the true uremias or with a group of nephritides designated by Strauss¹⁰ and by Volhard and Fahr¹¹ as pseudo-uremia.

In Table 2 are contained the estimation of similar waste products noted by others in patients with chronic interstitial nephritis in whom the diagnosis was confirmed by postmortem examination.

TABLE 2—ESTIMATION OF SIMILAR WASTE PRODUCTS IN CHRONIC INTERSTITIAL NEPHRITIS

Author	Number of Patients	Nonprotein Nitrogen Mg
Frothingham ¹²	36	45.7-257.7
Thayer and Snowden ¹³	26	50-210
Stengel, Austin and Jonas ¹⁴	5	*102-195 (uremia)
Strauss ¹⁵	19	68-266 (uremia)

* Urea nitrogen

All these patients had a marked increase in the nonprotein nitrogen of the blood during life and as a rule the amount was more than 100 mg per 100 c c.

The amount of nonprotein nitrogen in the blood in uremia is usually markedly increased but is subject to marked variations. Values ranging from 28 to 658 mg per 100 c c have been recorded by Strauss, Ascoli,¹⁶ Farr and Austin, Fitz and Rowntree,¹⁷ Widal,¹⁸ Obermayer and Popper,¹⁹ Hohlweg,²⁰ Foster,²¹ Tileston and Comfort²² and others.

10 Strauss, H. Ueber Uramie, Berlin klin Wchnschr **52** 368 1915

11 Volhard, F. and Fahr, Th. Die Brightsche Nierenkrankheit. Berlin 1914

12 Frothingham, C. The Relation Between Functional Tests and the Pathological Anatomy of the Kidney in Chronic Nephritis, Am J M Sc **151** 72 1916

13 Thayer, W. S. and Snowden, R. R. A Comparison of the Results of the Phenolsulphonephthalein Tests of Renal Function with the Anatomical Changes Observed in the Kidneys at Necropsy. Am J M Sc **148** 781, 1914

14 Stengel, A., Austin, J. H. and Jonas, L. A Comparison of the Functional and Anatomic Findings in a Series of Cases of Renal Disease. Arch Int Med **21** 313 (March) 1918

15 Strauss, H. Berl klin Wchnschr **52** 368, 1915

16 Ascoli, G. Pflüger's Arch f Path Anat **87** 103, 1901

17 Rowntree, L. G. and Fitz, R. Studies of Renal Function in Renal Cardio-Renal and Cardiac Diseases. Arch Int Med **11** 258 (Feb) 1913

18 Widal, F. Sem med **25** 313, 1905. Bull et mem Soc med d hop de Par **32** 627, 1911

19 Obermayer and Popper. Ztschr f klin Med **72** 332, 1911

20 Hohlweg, H. Deutsch Arch f klin Med **104** 216, 1911

21 Foster, N. B. Uremia. The Nonprotein Nitrogen of the Blood. Arch Int Med **15** 356 (March) 1915

22 Tileston, W. and Comfort, C. W. The Total Nonprotein Nitrogen of the Blood in Health and in Disease as Estimated by Folin's Methods. Arch Int Med **14** 620 (Nov) 1914

Of the various nonprotein nitrogen constituents of the blood, creatinin is the most uniformly increased in uremia, according to Folin and Denis,²³ and Myers and Lough.²⁴ Myers and Killian²⁵ found that in chronic nephritis an amount of creatinin in the blood exceeding 5 mg is of grave prognostic significance. Folin and Denis,²⁶ Myers, Fine and Lough,²⁷ Baumann and others²⁸ believe that uric acid is the first of the nonprotein nitrogen substances to be retained in nephritis.

SUMMARY

The patients in Groups I and II all had definite clinical, functional, chemical (and six patients, anatomical) evidence of nephritis. In the patients with both nephritis and myocardial inefficiency, the uric acid of the blood was in greater amount than in those with nephritis alone. Improvement of the cardiac decompensation was always associated with a decrease in the amount of uric acid in the blood. The average amount of uric acid in the blood of the patients in Group III with cardiac decompensation was considerably higher than that of the patients of Group II with chronic nephritis of moderate degree—the averages 6.9 and 6.06 mg per 100 c.c., respectively. It would appear, therefore, that greater uric acid retention is associated with passive hyperemia of the kidneys than with chronic nephritis of moderate degree.

The average amount of the uric acid in the blood of the patients of Group III with chronic nephritis was 4.53 mg, whereas that of the patients with myocardial inefficiency alone averaged 6.38 mg per 100 c.c.

The amount of albumin in the urine and the number of casts could in no way be correlated with the amount of the various nonprotein nitrogen constituents in the blood. Several patients with little evidence of nephritis in the urine had a moderate degree of nitrogen retention whereas a few with a large amount of albumin and many casts had little evidence of kidney insufficiency.

23 Folin, O and Denis, W. On the Creatinin and Creatine Content of Blood. *J Biol Chem* **17** 487, 1914.

24 Myers, V. C. and Lough, W. G. The Creatinin of the Blood in Nephritis. *Arch Int Med* **16** 536 (Oct.) 1915.

25 Myers, V. C. and Killian, J. A. The Prognostic Value of the Creatinin of the Blood in Nephritis. *Am J M Sc* **157** 674, 1919.

26 Folin, O. and Denis, W. The Diagnostic Value of Uric Acid Determinations in Blood. *Arch Int Med* **16** 33, (July) 1915.

27 Myers, V. C., Fine, M. S. and Lough, W. G. The Significance of Uric Acid, Urea, and Creatinin of the Blood in Nephritis. *Arch Int Med* **17** 570 (Nov.) 1916.

28 Baumann, L., Hansmann, G. H., Davis, A. C., and Stevens, F. A. The Uric Acid of the Blood as Compared with the Renal Dietary Test. *Arch Int Med* **24** 70 (July) 1919.

CONCLUSIONS

1 Chronic nephritis with hypertension and uremia is characterized by a marked increase in the amount of the nonprotein nitrogen substances in the blood and a low phthalein excretion

2 Chronic nephritis of moderate degree with hypertension is associated with a moderate increase in the amount of waste nitrogen in the blood and a lessened kidney function

3 Cardiac inefficiency without nephritis is associated with a moderate retention of the nonprotein nitrogen substances in the blood, more particularly the uric acid

4 In chronic nephritis with clinical and anatomic evidence of disease there is nitrogen retention and renal inefficiency

5 The presence of albumin and casts in the urine is not necessarily diagnostic of nephritis nor is their absence necessarily indicative of the nonexistence of such disease

6 Improvement of the circulatory disturbances is accompanied by a decrease in the various nitrogenous extractives of the blood, more particularly the uric acid and this may suggest that, at least, a part of the damage done the kidneys is a sequence of the alterations in its nutrition brought about by passive hyperemia

A CONTRIBUTION TO THE STUDY OF TUMORS FROM THE PRIMITIVE NOTOCHORD

N D C LEWIS, M D

WASHINGTON, D C

The notochord or chorda dorsalis of the amphioxus, in which it is the only axial skeleton, and in fishes and amphibians, in which it is still persistent in the axial skeleton, is of entodermal origin, while in the higher vertebrates the notochordal process and the mesoderm are apparently derived from the ectoderm. In developing embryos of the higher vertebrates, it becomes enclosed in the centers of the vertebrae and in the modified vertebrae forming the base of the cranium, in which sites it eventually and usually early in the mammals, degenerates, with the exception of residuals known as pulpy masses or nuclei pulposi located in the intervertebral discs.

The normal embryonic residual notochord tissue is composed of rather large, round or slightly oval, acidophilic epithelial cells which develop vacuoles containing mucin. In later stages, the cell wall disappears, thus freeing the cytoplasm which fuses with that of other adjacent cells, forming a syncytial structure which is not unlike that of fetal connective tissue. The adult notochord tissue is characterized by large cells containing vacuoles, eccentric, angular nuclei, and is bound together by a syncytial type of cytoplasm.

Abnormal growths of notochord tissue have most commonly been found at the spheno-occipital synchondrosis at the upper end of the notochord behind the pituitary body, and in the sacrococcygeal region, the lower end of the cord, although at least one has been described which occurred at the level of the upper cervical vertebrae¹. Many of the so-called chordomas are small benign masses of this tissue which has during early development been forced out of the bone, being probably analogous to other better known types of tissue displacement.

Virchow² termed these growths from the synchondrosis "ecchon-drosis physalifera" because of the vacuolated, degenerated cell surrounded by a bluish hyalin-like substance appearing somewhat like cartilage, and Mueller³ was apparently the first to be of the opinion that these small masses originate from rests of notochordal cells. These small chordomas, when growing in the cranium, have been

1 Klebs Allgemeine Pathologie, Pt 2, 693

2 Virchow Entwicklung des Schadelgrundes in den krankhaften Geschwulste

3 Mueller, H Ueber das Vorkommen von Resten der Chorda dorsalis beim Menschen nach der Geburt und uber ihr Verhaltnis zu den Gallert geschwulsten am Klivus, Ztschr f rat Med 2 202, 1858

known to assume dimensions great enough to produce symptoms of intracranial pressure,⁴ but usually, as shown by Ribbert's⁵ investigations on these tumors of the region of the clivus, the growths are small and soft, hence do not produce clinical manifestations, are connected by the slender pedicle with similar tissue imbedded in the bone, perforate the dura, and often are attached to the basilar artery. He stated that these small nodules, to which he gave the name chordoma, were found in 2 per cent of all necropsies.

I have recently removed at necropsy one of these soft pale gelatinous, pea-sized tumors from the clivus blumenbachii. This small growth was attached by a pedicle to a similar structure imbedded in the bone and had perforated the dura. Microscopic examination revealed a pale, basic staining cartilaginous appearing matrix in which there were a few large vacuolated cells with marginal nuclei (Fig 6—C).

Fischer⁶ first described the malignant chordoma occurring at the base of the skull, and malignant, invasive chordomas of the sacrococcygeal region have been described by Mazzia,⁷ Albert⁸ and others.

Because of the peculiar vacuolization of cells, the arrangement and staining properties of the matrix and invasive mode of extension, the differentiation of chordoma, myxochondroma and colloid carcinoma is often difficult, and doubtless many errors have arisen in this connection, particularly when the growth has occurred in the sacral region. The few differentiating features will be discussed at the end of this paper.

With the exception of Albert's case, I am not aware of any written reports of these cases in this country, and as instances of malignant chordoma are rare, their pathologic characteristics are not well known, and as four are now available for analysis it seems desirable to publish such an account.

Case 1 came to necropsy in our laboratory at Saint Elizabeth's Hospital. Case 2 was seen by Lieutenant Commander John Harper, U S Naval Medical School. Cases 3 and 4 were brought to my attention by Dr. Joseph C. Bloodgood of Johns Hopkins University, who kindly gave permission and material from the records for their publication.

4 Grahl. Eine Eechondrosis Physalifora Spheuo-Occipitalis Ungewohnlichen Umfanges, Dissertation, Gottingen, 1903.

5 Ribbert. Zentralbl f path Anat, p 149, 1894, Geschwulstlehre, p 149, 1904.

6 Fischer. Ueber ein malignes chordom der Schadelruchgratshohle, Beitr z path Anat u z allg Path, Jena 40 1907.

7 Mazzia. Chordom der Sakralgegend, Zentralbl f allg Path u path Anat 21 769, 1910.

8 Albert, H. Chordom, with the Réport of a Malignant Case from the Sacrococcygeal Region, Surg, Gynec & Obst 21 776, 1915.

REPORT OF CASES

CASE 1 (1921) —Male, white, aged 54, born in Ireland, soldier, stated that his habits were always good. He never drank to excess, worked regularly and had always been in good health until the age of 23 when he had an acute mental upset which led to his arrest and commitment to the Government Hospital for the Insane, where he remained until his death.

Family history not available.

Physical Examinations at the time of admission and during the following ten years showed no abnormalities. Mentally he was of the paranoid constitution, admitting occasional auditory hallucinations, frequently becoming restless and excited, walking up and down the ward talking angrily to himself in profane and vulgar language.

Present Illness —Six years ago the patient was described as being neat in dress and habits, sleeping well, and apparently in good physical condition with the exception of "lumbago" pains and external hemorrhoids. A Wassermann reaction at this time was weakly positive, and probably without significance. During the next year there was mentioned that the patient was frequently violently angry at some of his associates and was engaged in several ward fights (possibility of coccygeal injury). A year before death, he had a parole, did fairly efficient work in the dining room, but had a few rather insignificant delusions. His physical health was recorded as satisfactory.

Four months before death it is stated that he was suffering from diarrhea and tenderness of the abdomen. A month later, he complained of gastrointestinal pain, nausea, diarrhea and headache. A large nodular mass surrounding the rectum and bladder, the functions of which were intact, was palpated in the pelvis. There was sufficient abdominal and pelvic pressure to produce severe alterations in the circulation of the thighs and scrotum, which were edematous and cyanotic. Palpation of the body surface showed a board-like rigidity of the inner portion of the left thigh, and of the abdomen as high as the umbilicus. There was considerable induration about the testicles.

Diagnosis —A diagnosis of inoperable pelvic malignancy was made.

Clinical Course —The patient gradually became weaker and more emaciated until death supervened, a little over three months after acute symptoms were noticed.

POSTMORTEM FINDINGS

External Examination (twenty hours after death) The subject was a middle aged man with a slender skeleton and a large type of skull, the body was considerably emaciated, but showed residuals of former powerful muscles. There was a diffuse bluish-yellow cast to the skin. The entire abdomen below the umbilicus was extremely firm and rigid, as was also the area over the adductor group of muscles of the left thigh, as outlined in Figure 1. The boardlike resistance of this area gave one the impression of an ossifying myositis.

The penis was edematous and the testicles were drawn tightly against and attached to the os pubis to the extent of complete immobility. The perineum was also resistant to pressure.

The skin covering these areas was unbroken and evinced no pathologic changes.

Internal Examination On section of the abdomen, the parietal peritoneum from the level of the umbilicus downward into the bladder region was thick, white, glistening, slightly roughened, extremely fibrous, and had the consistence of cartilaginous structure. Near the pubic bone, the peritoneum averaged from 8 to 10 mm in thickness, gradually thinning out toward the umbilicus. The landmarks of the pelvic and dorsal peritoneum were completely obliterated by solid continuous sheets of tumor invasion.

Tumor —The principal mass was now located and found to fill the entire pelvis proper, enclosing the rectum, bladder and other pelvic viscera in a firm encasement. Its broad attachment to the entire sacral curvature from near

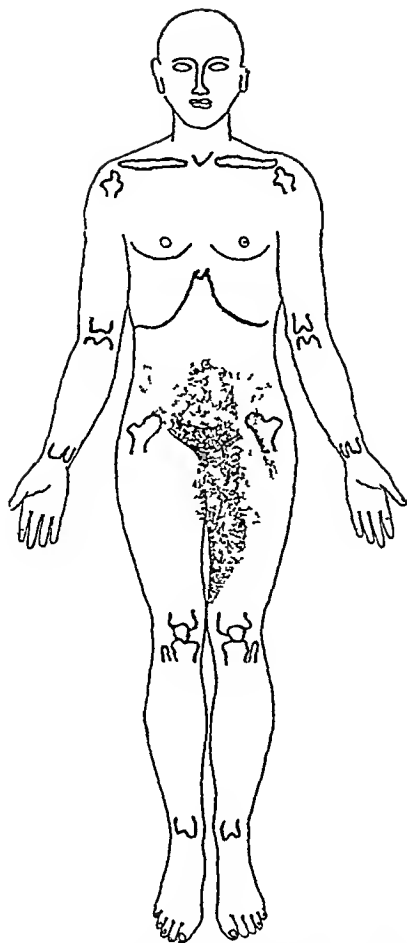


Fig 1—The shaded area outlines the extent of surface rigidity



Fig 2—Section of sacrum and coccyx showing the broad attachment of the principal tumor mass to the curvature

the promontorium to and surrounding the tip of the coccyx is illustrated in Figure 2. Gross sections of the main mass revealed a whitish, firm, melastic tissue in which small orange-colored fat accumulations were entrapped.

A large fossa was produced in the tumor by the laborious removal of the bladder and rectum. The bladder was sacculated in a long conelike extension at the apex, compensatory to the extreme rigidity and immobility of the remainder of its walls. The ureter was intact and the mucosa of the entire structure exhibited pressure changes and punctate hemorrhages, but no actual erosions.

Prostate—The prostate gland was under considerable pressure from the surrounding growth which had completely filled the capsule, but had not entered the glandular substance (Fig 3, B).

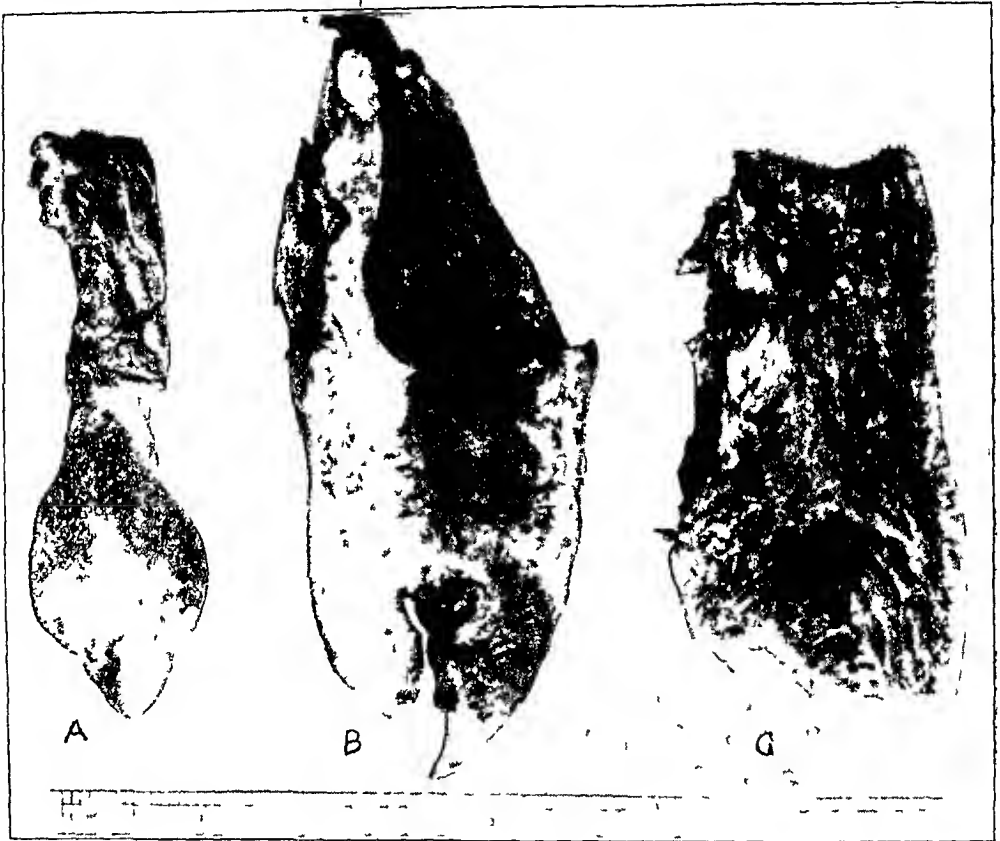


Fig 3—A, testicle and cord surrounded by solid tumor growth, B, section of infiltrated bladder and rectal walls with prostate between, C, section of the skin and thickened subcutaneous tissues of the thigh

Rectum and Sigmoid—The walls of the rectum and sigmoid were completely infiltrated with foreign growth as far as the mucosa, which showed hypertrophic and advanced hemorrhagic changes, the lumen of the gut was open throughout but distorted. The entire gut, including the sigmoid and lower portion of the descending colon was almost inflexible and reminded one of stiff garden hose. The mesenteries of the colon and small intestines presented a mild but distinct increase in texture, and in patches were tightly attached to the abdominal and pelvic fascias, allowing very little mobility to the intestinal tubes. The growth extended through the mesenteries in a thin sheet with no nodules and without infiltrating the lymph glands, which were normal in size and color. The infiltration of the tumor extended upward as high as the trans-

verse colon but had not affected the walls or attachments of the stomach. The walls of the small intestines were not grossly infiltrated, and the mucous membranes were normal in appearance.

Liver—The undersurface of the diaphragm, the capsule and ligaments of the liver were not affected. The liver exhibited a diffuse edematous, toxic, congested appearance, with a moderate central lobular degeneration. The gall-bladder was small but contained normal appearing bile.

Spleen—The spleen was imbedded in a thick, firm, gray capsule of foreign growth, which was not nodular but slightly roughened and enclosed the organ like a case. In general, it stripped easily from the surface, but firmly adhered to the capsule in patches. The substance of the spleen presented productive interstitial changes, but no gross invasion of the tumor.

Pancreas—The pancreas was surrounded by sheets of tumor growth, which had followed the connective tissue and vessels into the gland, ramifying profusely between the lobules which were affected only by pressure changes.



Fig 4—A, section of the kidney showing thickened capsule, infiltration of pelvis, and imbedding of adrenal in chordoma extensions, B, kidney capsule diffusely infiltrated with chordoma.

Kidneys—Both kidneys were encased by neoplastic growth which had fused with and stripped along with the capsule, leaving the surface of the organs corrugated from the prolonged effects of diffuse productive nephritis. Sections exhibited congestion and acute changes through the cortex. The tumor had extended along the ureters and vessels in the pelvis of the organ with extensions through the peripelvic fat and about the papillae (Fig 4). The ureters were surrounded by the tumor, and the walls were infiltrated, but the lumina were patent.

Suprarenals—The suprarenal glands were deeply imbedded in the tumor mass (Fig 4), and showed exhaustion of the medulla, and many minute cortical hypertrophies.

Pelvic Fascia—Extending from the sacral area through the pelvic fascias, which in places were half an inch in thickness, the tumor spread over the left Poupert's ligament and down the subcutaneous tissues of the inner portion of the left thigh nearly as far as the knee, crowding the adductor group of

muscles beneath it, but apparently not infiltrating them. In places, these subcutaneous tissues were $1\frac{1}{2}$ inches in thickness, grayish in color, and of cartilaginous texture mixed with fat (Fig 3 C). The subcutaneous tissues of the outer portion of this thigh and the fascia lata were normal in appearance. The tumor had not extended below Poupart's ligament of the right side, but had grown centrally into the tunics of the testicles, completely surrounding but not infiltrating the glandular substance, although radiations could be traced into the mediastinum testis. There was apparently, in addition to the actual tumor, a tremendous fibrous tissue response, so that the structures were bound tightly to the pubic bone. The spermatic cords with their structures and extensions were heavily infiltrated, firm, rounded and had attained the diameter of one-half inch (Fig 3 A).

Lungs—Detailed examination of the thoracic viscera revealed neither evidence of tumor extension above the diaphragm nor gross metastases.

The lungs were both free in the pleural cavities, with the exception of a few well organized adhesions at the left apex, and the upper lobes were collapsed. Section revealed normal upper lobes, while the lower lobes of both organs showed a moderate congestion and edema without consolidations. The mucous membranes of the trachea and bronchi appeared somewhat anemic.

Thyroid—The thyroid revealed a reduction in the amount of colloid and advanced interstitial changes.

Heart—The heart was in normal position, and the pericardium was not remarkable. The muscles were rather pale in color and the cardiac veins considerably distended. In general, the organ was normal considering the age with valves in good condition and only a slight interstitial myocarditis. Aorta was normal with the exception of a mild fibro-fatty plaque production.

Skull—The skull was thin throughout but the parietal bones were extremely so. The dura mater was slightly adherent to the calvarium and thickened to the extent that the brain convolutions were not easily visible through it. The cerebrospinal fluid was apparently normal.

Brain—The brain weighed 1,200 gm and was remarkably anemic, the larger veins being empty and the smaller capillaries barely visible. The brain substance was universally edematous, and the arteries of the base showed a few discrete arteriosclerotic nodules, but otherwise there were no gross abnormalities.

Pituitary—The pituitary gland with the sella turcica was not unusual.

MICROSCOPIC EXAMINATION

Sections from Sacral Tumor—The connective tissue response through these sections was remarkable and large bands of this structure were filled with the characteristic cells of malignant notochordal tissue. In many places there were localized groups of large vacuolated cells, bearing eccentric nuclei of various sizes, and lying in a pale, finely granular matrix, while in other fields these cells were thinly scattered through the fibrous tissue.

In some areas there were many variations in the cell structures and type of arrangement. The classification I offer is descriptive of the types frequently encountered (Fig 5).

1 Cells with thin rim of finely granular protoplasm, a single large central vacuole which has often ruptured the enclosing protoplasm, and a marginal nucleus which varies in size, staining properties, and is often multiple (b).

2 Cells with a heavily stained, irregular nucleus only slightly eccentric and with relatively more protoplasm bearing several large sized vacuoles (a).

3 Cuboidal, polyhedral or columnar cells with central irregular nucleus and protoplasm with a few small vacuoles (c).

4 Large cuboidal or columnar cells with a narrow, marginal, irregular, heavily stained nucleus, and relatively large amount of protoplasm containing a few small vacuoles (d).

5 Slightly irregular cuboidal, polyhedral or rounded cells bearing a relatively large granular nucleus and without vacuoles in the protoplasm (e)

6 Irregular and compound cells with peculiar arrangements of the nucleus and protoplasm—formed probably by fusion of cells after partial discharge of mucem or by cell division under pressure (f)

Sections of Skin and Subcutaneous Tissue—The skin layers, the hair follicles, sebaceous and sudoriferous glands were apparently normal, however, about some of the lower sweat glands, chordoma cells were seen proliferating, often lying up tightly against the basement membrane. The fibroblastic and fatty elements of the subcutaneous tissues were enormously increased and diffusely infiltrated by the foreign cells, bunches of which were accumulated

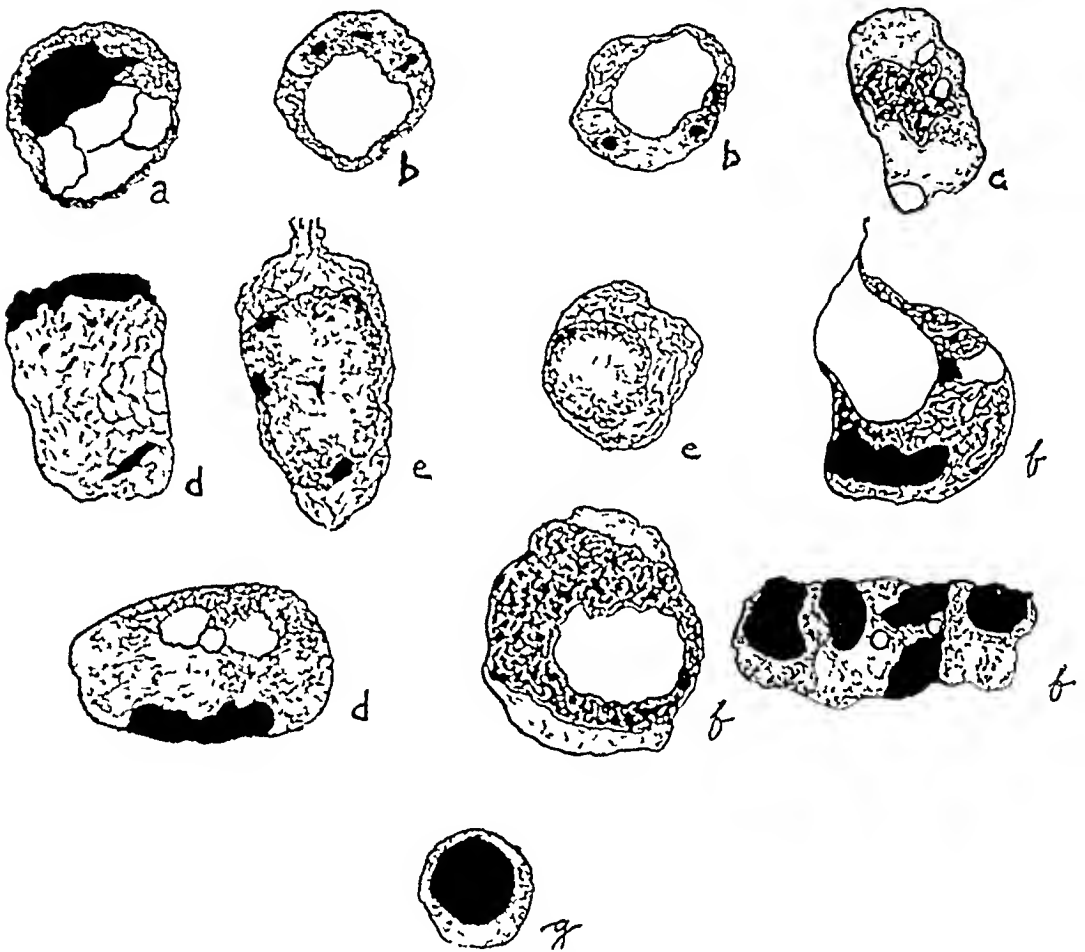


Fig 5—Types of cells seen in malignant tumors of the notochord (drawn to same scale) (a) Cells with heavily stained, irregular nucleus only slightly eccentric and with relatively more protoplasm bearing several large sized vacuoles (b) Cells with thin rim of finely granular protoplasm, a single large central vacuole which has often ruptured the enclosing protoplasm, and a marginal nucleus which varies in size staining properties and is often multiple (c) Cuboidal or columnar cells with central irregular nucleus and protoplasm with a few small vacuoles (d) Large cuboidal, columnar or polyhedral cells with a narrow marginal irregular heavily stained nucleus and relatively large amount of protoplasm containing a few small vacuoles (e) Slightly irregular, cuboidal polyhedral, or rounded cells bearing a relatively large granular nucleus and without vacuoles in the protoplasm (f) Irregular and compound cells with peculiar arrangements of the nucleus and protoplasm, formed, probably, by fusion of cells after partial discharge of mucem or by cell division under pressure (g) Small lymphocyte for comparison

in the more areolar portions of the tissue. Deep in the tissues, near the muscles, there was a notable lymphocytic reaction with quite dense fibrosis of the structures. In these deeper layers the chordoma cells showing multilobulated nuclei, sometimes central, generally eccentric and occasionally scaphoid, and always situated in a relatively large amount of pale blue protoplasm, were in small groups numbering from three to eight cells per group.

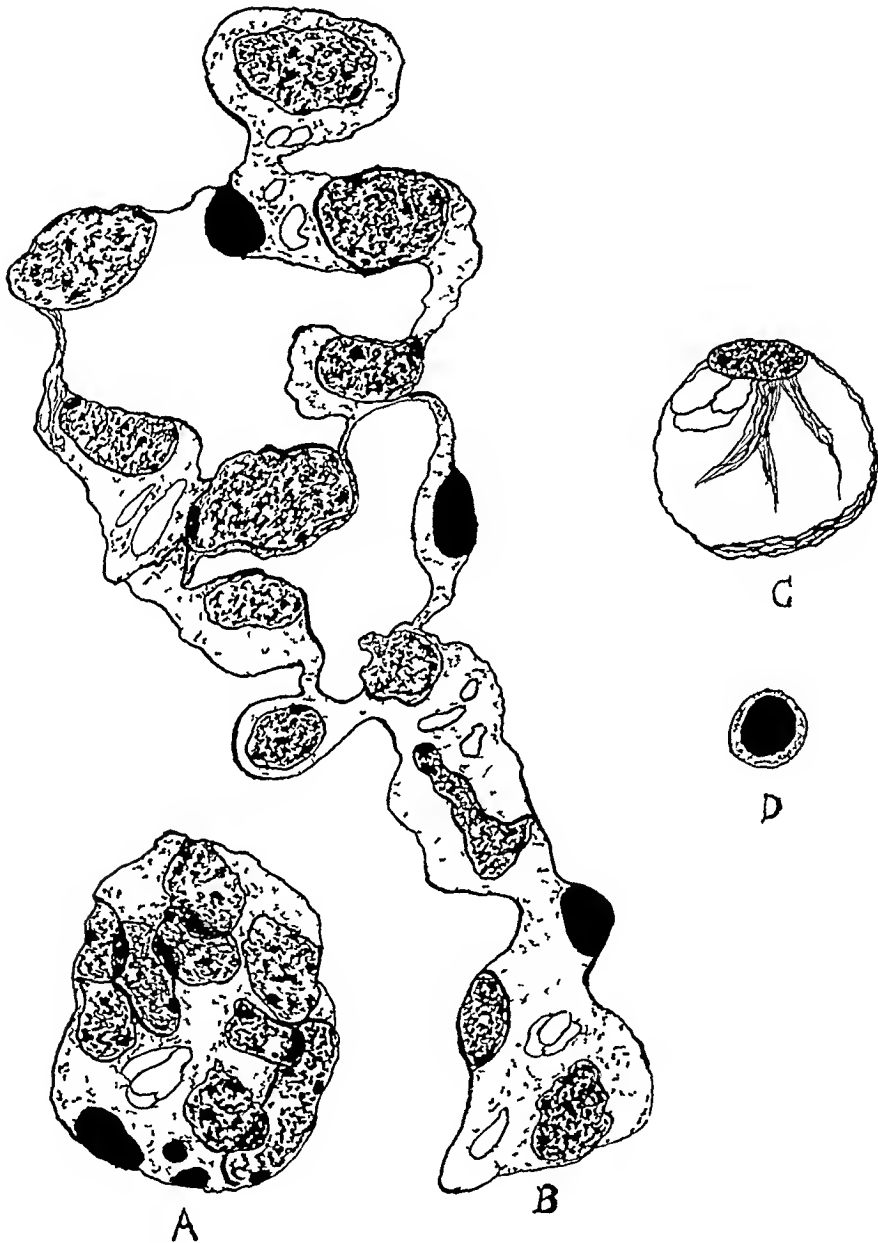


Fig 6—A, nests of chordoma cells as seen in mesenteries and tunica vaginalis of the testicles, B, type of cell arrangement seen in all four cases of chordoma—fusion of cell walls, discharge of mucus, and formation of vacuolated syncytial like structure, C, type of benign chordoma cell, D, small lymphocyte for comparison

The blood vessels of this area were thickened but nowhere were foreign cells seen penetrating the walls, but doubtless many of the smaller arterioles were obliterated by extensions of cells. One large subcutaneous artery was surrounded by chordoma cells, but the walls proper were not affected. The

accompanying nerves with their sheaths were also surrounded but not infiltrated, however, in some sections of the compound fasciculae, a few chordoma cells were seen between the nerve bundles in the epineurium

The small regional lymph glands showed a dense obliterative central fibrosis and an irregularly thickened capsule from which extensions radiated into the glandular tissue greatly reducing the lymphoid elements. The surrounding tissues were filled with malignant chordoma cells but none had entered the lymph glands



Fig 7 — Photomicrograph of section of rectal wall in Case 1, showing large vacuolated cells with eccentric nuclei separating bundles of muscle and connective tissue

Sections of Mesenteries—There was a fibrous tissue increase in wide bands throughout, with many large areas of fat cells in which were seen accumulations of rapidly dividing chordoma cells arranged in nests (not unlike carcinomatous formations) often containing as many as twenty-five or thirty cells (Fig 6 A)

The lymph gland capsules were enormously thickened, fusing with the surrounding structures, and were infiltrated with actively growing tumor cells, but the glands themselves, although they exhibited considerable increase in stroma

were not infiltrated by these cells, all extensions of which appeared to stop at the inner capsular margin

Walls of Rectum—The walls of the rectum showed a dense infiltration of all coats with chordoma cells of both the lower embryonic type, and of the older vacuolated eccentric nucleated variety which extended along the fascias between the muscle bundles (Fig 7) and into the submucosa. The mucosa was not remarkable with the exception of occasional small sized necroses probably produced by pressure changes

Prostate—The prostate gland presented a universal adenoplasia of first degree, with irregularity of the acini and hyperplasia of cells, and with considerable concretion deposits in the centers of the follicles. There were no tumor cell extensions in the vicinities of the prostatic glands, with the exception of an occasional small patch of widely separated cells usually situated near one of the acini, but in the fascias surrounding the prostate and between it and the bladder walls and rectal walls there were both nested groups and diffuse infiltrations of chordoma elements of the darkly stained, large nucleated types in a matrix having a high mucin content

Testicles—The testicular acini were characterized by a thickened basement membrane, with the attached spermatogenic cells greatly reduced in number and with no spermatozoa. The interstitial tissues were sclerotic throughout. The tunica vaginalis and tunica albuginea were fused and enormously thickened. The tumor infiltration in these regions was patchy in distribution, and in places nests of small dark nucleated chordoma cells, surrounded by clear spaces, gave the general appearance of carcinomatous arrangement, however the peculiar pale staining mucinous matrix was differential. Neither the acini nor the interstices of the glands proper had been attacked, but the process was limited to the envelopes of the organ, and extended along the spermatic cord following the fascias, and widely separating its structures, principally from the associated tremendous fibrous tissue production

Sections of Kidney—(Description common to both kidneys) The capsule of the organ was greatly thickened, being a dense, almost solid mass of chordoma cells which filled the entire capsule and were lying close to the glandular margin but had not dipped down between the cortical structures excepting where the capsule had been followed. The tumor cells of this region were in a connective tissue stroma and representatives of all cell types of Figure 5 were noted

The outer zones of the kidney cortex were densely sclerotic and in patches only the knotty remains of tubules and glomeruli were seen. Many convoluted tubules were hyperplastic with a slight granular exudate in the centers. Deeper in the cortical structures, the glomeruli were somewhat swollen and hemorrhagic, and the tubules were distorted, the cells which were originally flattened exhibiting advanced albuminous degeneration. In a few areas dense intratubular and extratubular hemorrhages were noted, and the stroma, in general, was increased throughout the organ

Suprarenals—The tissues surrounding the glands had fused with those of the kidney, and contained many thickened vessels and wide zones of fibrosis with a large amount of fat and areolar tissue

Scattered discretely through these tissues, and particularly in the areolar areas were both isolated and small accumulated groups of tumor cells, without infiltration of the suprarenal cortex. The cortex of the gland showed considerable atrophy with a few localized compensatory glandular hypertrophies. The zona glomerulosa remained only in a few marginal knots, and the lower zones appeared to be degenerated and exhausted. The medullary portion was heavily sclerotic, narrowed and presented much lymphatic infiltration

Liver—The capsule was thin but fibrous, and showed no evidence of infiltrating tumor cells. There were no tumor cells through the sections taken from the organ but the substance exhibited diffusely scattered blood pigment and an irregularly distributed fatty degeneration extending through the lobules and about the central lobular veins

There was a numerical reduction in the liver cells, and the remaining ones were swollen, pale and often vacuolated. A moderate increase in connective tissues was noted in the portal canals.

Pancreas—Through most of the organ there was a dense sclerosis with complete atrophy of about half the acini, the remaining ones being bunched in small groups and presented heavily stained cells. The islands were distorted and sclerotic showing a reduction in the number of cells. In the fascias about the gland many irregular groups of chordoma cells, held together by a mucinous stroma, were surrounded by fibroblastic productions. These tumor cells had followed the interlobular stroma for short distances into the gland, but in no instance were the tubules involved, excepting perhaps by pressure.

Spleen—The capsule was thickened, sending ramifications of large size into the substance of the organ, and contained considerable dark brown pigment,



Fig 8—Photomicrograph of chordoma cells scattered among the connective tissues and fat cells of the mesenterics (Case 1)

but there were no chordoma cells in the sections studied. There was a general increase in splenic stroma, particularly about the vessels, the malpighian bodies were small and the arterioles were thickened and occasionally hyaline.

Lungs—No tumor metastases were seen in the lungs, which were not remarkable, with the exception of some congestion of the alveolar arterioles and a peribronchial round cell infiltration.

Thyroid—The interfollicular structures were filled with epithelial and lymphoid cells and the colloid was diminished to about one-half the usual amount. Wide bands of connective tissue excessively lobulated the organ.

Heart—The muscle fibers showed some separation and replacement, the muscle cells were pale and atrophied, bearing pale granular nuclei, at the poles

of which was a large amount of brown pigment. An occasional large endothelial cell was noticed between the muscle fibers.

Cerebral Cortex—There were no unusual changes, with the exception of mildly edematous perivascular lymph spaces and some thickening of the overlying meninges.

Cerebellum—The meninges covering the leaflets and extending into the fissures showed fibrosis and some endothelial hypertrophies. The architecture of the cerebellar layers was not remarkable, but there was some reduction in the number of purkinjean cells, with a neuroglial felting of the areas.

Spinal Cord—The cord presented no striking changes, but there was some overlying chronic meningitis and an unusually large number of modified ependymal elements in the central canal region.

Section Including Pelvic Sympathetic Ganglia—Large patches of chordoma cells were present in sections in which the associated connective tissue response was much less in evidence.

The sympathetic ganglia were surrounded by, but not infiltrated with, tumor cells. Many of the ganglion cells were shrunken, heavily pigmented and surrounded by large pericellular spaces. Some of these cells were acidophilic in reaction, and a few were completely disintegrated.

Pituitary—The posterior lobe showed dense sclerosis and considerable brown pigmentation, while the anterior lobe was only moderately sclerotic, and the acini were large, well stained and in a good state of preservation.

CASE 2 (U S Naval Hospital)—A male, white, aged 30, with negative family, past and venereal histories, was admitted to the hospital June 2, 1919, with a history of persistent constipation, with no passages of feces for five days. Vomiting started twenty-four hours after admission, and because of increasing abdominal symptoms a laparotomy was done June 4. All intestines were found to be dilated with fluid, and an annular mass about one inch in length, and of the thickness of a thumb was found obstructing the sigmoid. This mass, with the sigmoid, was fixed to a "stab wound" of the left side and resected four days later (artificial anus). The tumor was diagnosed as carcinoma.

A second laparotomy was done Oct. 20, 1919, which showed the large intestine and peritoneum to be covered with small nodules, which were clinically considered to be either carcinoma or tuberculosis. The pathologic diagnosis from sections of the peritoneal nodules was sarcoma.

Nov. 4, 1919, an operation was done for rectal fistula, which had formed, with resection of the lower rectum, and another laparotomy again showed many nodules over the omentum, but the tumors seemed smaller and less numerous than at the previous operation.

Death occurred Feb. 25, 1920, with clinical evidence of intestinal obstruction. There was a very slight emaciation of the body and tumor masses were palpable through the abdominal wall.

NECROPSY

A necropsy was performed, and the parietal peritoneum, the omentum, the mesenteries and the intestinal walls were studded with small nodules like those seen at the operations. The intestinal obstruction had been caused by a large mass of tumor about 6 inches through, involving the omentum, descending colon and sigmoid. Several associated smaller masses were noted, with enlargement of the retroperitoneal and mesenteric lymph glands.

The liver was congested and exhibited small nodules of tumor tissue in the capsule but none in the substance, and there were no metastases into the thorax.

Microscopic Findings—Sections taken from the wall of the resected rectum showed the muscle bundles to be separated by round and cuboidal, rapidly multiplying cells, having clear vacuolated protoplasts, and deeply stained, irregular eccentric and often multiple nuclei, surrounded by an abundance of chronic inflammatory tissue. Sections including the mucosa presented no

remarkable changes in this structure nor in the immediate submucous tissues, but the tumor infiltration was rather limited to the muscular layers. No malignant changes were noted in the mucosa or skin of the mucocutaneous junction at the anal opening.

Sections of the intestinal walls taken at the necropsy gave the impression of a more rapid growth of the tumor than those taken previously at operation. The streaks of malignant cells had split the muscle fibers into narrow fine strands of tissue, which were atrophied, forming a stroma in places around the individual cells or small groups of cells, although, in general, there was very little tendency to the type of grouping seen in Figure 6 (A and B). A large majority of the cells were closely packed and of the large vacuolated variety with eccentric nuclei (Type 1 of classification), with less surrounding connective tissue reaction than in the earlier sections, and apparently rapidly extending (Fig 9). None of the larger vessels were penetrated by the growth.



Fig 9—Photomicrograph of section of intestinal wall from Case 2 demonstrating rapid infiltration of chordoma cells in the muscular layers

All the tissues examined from this case were of the above type of reaction, and no evidence of carcinomatous changes or tuberculous lesions was discovered.

CASE 3 (Case of Dr. Riggs, South Dakota)—White, male, aged 58 merchant with a negative family history, and a personal history which was negative until two years before operation, at which time he complained of frequent urination with burning. Cystoscopic examinations were negative and the condition was relieved by passing urethral sounds.

The patient entered a hospital in May, 1920, complaining of pains in the rectal region, the right leg, and also in the arms, and he felt chilly. He remembered that during the past two years he had had some discomfort about

the rectal region, occurring usually in the night, and relieved by changing the position. His condition was diagnosed as ischio-rectal abscess.

Physical Examination—This revealed a normal chest, abdomen and prostate. The tonsils were small and "positive." Blood pressure, 88-92/60.

Dorsally, at the lower portion of the coccyx was a mass about the size of a prostate, composed of two distinct nodules, slightly movable, apparently not attached to the rectum, and of the consistence of fibrolipoma.

Treatment—The tonsils were removed. The blood pressure became better (110), and the tumor was excised.

Tumor—The growth measured 6 by 5 cm in two diameters, was enclosed in a definite but thin capsule (Fig 10), and contained one piece of bone suggesting the tip of the coccyx. The tissue seemed very cellular, with a fine stroma, and was dark brown in color, with gray and white areas. The substance was friable, and some small granular, elevated masses were easily picked from the tissue. The structure did not suggest chordoma or cancer in a dermoid, but was not unlike a giant cell tumor belonging to the type of malignant sarcoma.



Fig 10—Gross sections of the chordoma removed from the rectal region in Case 3.

Microscopic Examination—This revealed a very cellular structure, with a tendency to alveolus formations, with separations by wide dense strands of eosin staining, hemorrhagic, fibroblastic tissue, which in places included small groups of cells. The cells rested on this and grew out in capillary masses, with fusion of cytoplasm and exudation of mucin, but with no definite intercellular substances. The cells in the center were undergoing disintegration leaving mucin and an eosin staining debris.

The predominating types of cells were cuboidal and round with a lightly staining protoplasm, while the nuclei were large, granular, centrally situated (Fig 5 E), and showing many forms of karyokinetic figures. There were a few areas showing vacuolated cells bearing the half moon eccentric nuclei typical of chordoma, there were also a few multinucleated cells of giant cell dimensions.

CASE 4 (Sent in by Dr Bassett, Savannah Ga.)—In September, 1914, a white female aged 22, applied for relief on account of constant pain in the rectal region. Otherwise she was in good condition, showing no loss of weight. Two years before a surgeon had excised a portion of a rectal tumor, which he diagnosed as cancer.

Rectal Examination—A small hard nodular, irregular mass about 2 inches long was situated at the left side of the rectum and attached to the pelvic fascia, but was not attached to the wall or mucous membrane.

The rectum was incised and a specimen removed. The condition at this time was thought to be quite independent of any previous operation. The possibilities of carcinoma, endothelioma and tuberculosis were considered. The specimen removed for examination was a small piece of firm, white tissue resembling a gland, and at the time was thought to be probably of epithelial origin.

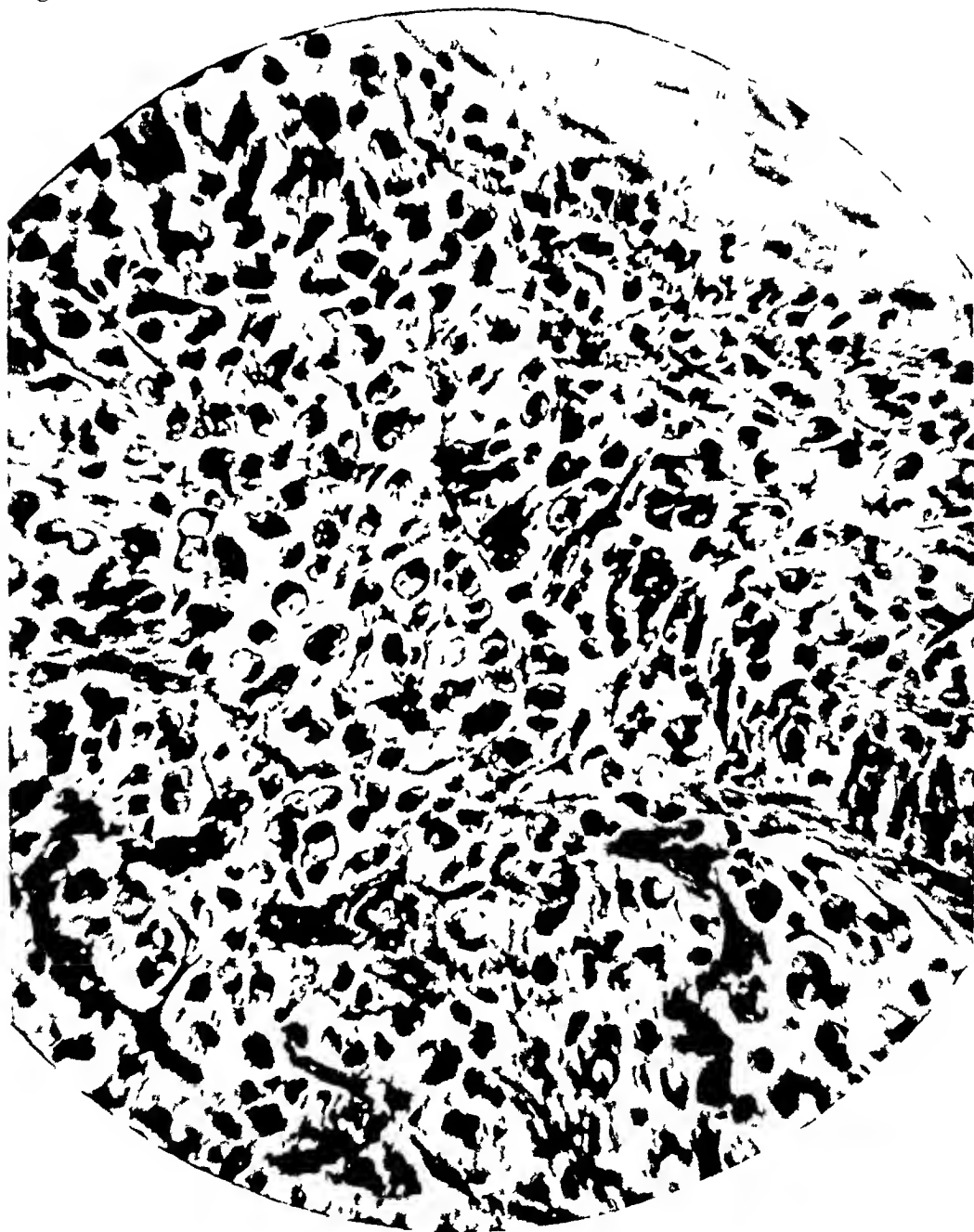


Fig 11—Photomicrograph of section taken from tumor metastasis in cervical region in Case 4, illustrating the varieties of cells characteristic of malignant chordoma.

About a year later (October, 1915) the tumor recurred at the rectum with complete obstruction, necessitating opening of the sigmoid above (artificial anus). The patient was now considerably emaciated, and a lump the size of an apple (probably metastasis) had grown in the neck.

Microscopic Examination—The specimen removed from the rectal region showed a few irregular masses of dense bone, in the meshes of which malignant vacuolated cells of polyhedral and round outlines, with eccentric nuclei, were distributed. These cells resembled those seen in the other three cases, but, on the whole, were somewhat smaller, and showed a pronounced tendency to small groupings in dense connective tissue. The stroma of this tumor was abundant, and many areas of areolar tissue were filled with ringlike cells.

The blood vessels were all thickened, but not, as a rule, penetrated, although in one instance chordoma cells were noted growing in the walls and in the central portion of a thrombosed artery.

At death there were several other masses in the cervical lymph glands, the size of large walnuts. A necropsy could not be obtained, but a tumor was taken from the neck for diagnosis.

The microscopic characteristics were like those of the original tumor from the rectal area, with the exception of being more cellular and with less connective tissue production, and sections exhibited the various types of cells described above for the other tumors and are well illustrated in Figure 11, a photomicrograph from the tissues of this case.

CONCLUSIONS

1 In the majority of cases of chordoma reported the tumor occurred in the spheno-occipital region, so that it is rather singular, but fortunate, to have four instances of this type of malignancy growing from the sacrococcygeal site, giving an opportunity for comparative studies.

2 There is no history suggesting the etiology in Cases 2, 3 and 4, but in Case 1 there is a strong probability of injury to the coccygeal area, and as this region is the site of frequent trauma, the possibility of injury as a causative factor must be considered. (A definite history of traumatism was obtained in the case reported by Albert.)

3 Comparative Summary. In Case 1 the symptoms (diarrhea, nausea, abdominal tenderness and headache) became severe enough to attract attention only four months before death, during which time emaciation and cachexia developed rapidly. This patient was an insane man, in whom subjective symptoms may have been and probably were present long before his condition was complained of or came to the attention of the physician.

A complete necropsy was performed which revealed the principal tumor broadly attached to the sacrum and coccyx, from which site extensive invasions, associated with tremendous connective tissue productions, had radiated through the fascias, capsules and walls of the abdominal organs without infiltrating glandular structures or affecting mucous membranes, except by pressure. The inhibiting effects of this diffuse growth on the functions of the abdominal viscera must have been considerable. There were no true metastases nor nodular formations in the path of the tumor.

In Case 2 death occurred about eight months after the onset of acute symptoms (persistent constipation, vomiting, increasing abdominal symptoms). Early laparotomy showed obstruction of the sigmoid,

and a later laparotomy revealed many small nodules in the peritoneum and in the large intestine. A rectal fistula formed, which was corrected, and a third laparotomy showed many nodules over the omentum.

In this case the emaciation of the body was slight. A necropsy was performed. No mention was made of a tumor attached to the sacrum, but a large mass was described involving the omentum, sigmoid and descending colon, as well as a general distribution of many small nodules in the peritoneum, mesentery and intestinal walls. This feature of growth was quite different from that observed in Case 1.

The earlier sections from Case 2, taken from the resected rectal wall, exhibit a liberal connective tissue response with relatively few chordoma cells, while those taken from the intestinal walls at necropsy were rich in tumor cells and with less connective tissue—showing, in general, a more rapid growth, as in Case 1 no metastases were seen in the thorax.

One patient (Case 3) gave a history of rectal discomfort for two years before the acute symptoms (chilliness, rectal pain, pains in legs and arms) began. A slightly movable, irregular, mass was palpated at the lower portion of the coccyx. This mass measuring 6x5 cm was excised and found to correspond microscopically with the characteristics described for chordoma. The operation was performed about a year ago. The present condition of the patient is not known to me.

The last patient (Case 4) sought relief from acute symptoms (constant pain in rectal region) a little more than a year before death. At the time of the acute onset she was apparently in good general physical condition, but rectal examination revealed a small, firm, nodular tumor outside the rectal wall in the pelvic fascia. A year later, the tumor in this area produced complete obstruction of the rectum necessitating sigmoid resection (artificial anus). The patient then showed emaciation, and metastases were forming in the cervical region. Death followed, but no necropsy was performed, although a nodule was taken from the neck for diagnosis.

The cells and general structure of the metastasis were those of the original rectal tumor.

It is regretted that a necropsy could not be obtained in this case, as apparently there was a true metastasis to the neck, and it would have been instructive to observe the mode of extension, if any, through the abdominal viscera.

4. So few of these tumors have been recognized and reported that one hesitates to offer an opinion as to their frequency, but that they are of far more clinical importance than has heretofore been considered is quite evident, and judging from the variety of diagnosis made in the four cases cited by as many competent pathologists and surgeons, these tumors are much more frequent than was formerly

supposed, and they have undoubtedly occasionally been classified among the other forms of malignancy

A differentiation of chordoma from myxochondroma and from colloid carcinoma of the rectal region is not readily accomplished, as the types of cells are often similar, and many other features, such as mucein content, cell formations and stroma arrangements are seen in common. The absence of actual cartilaginous formations speaks against myxochondroma, and broad attachments to the sacrum, with extensive infiltrations through the supporting tissues, and at the same time exhibiting a tendency of limitation to the capsules of organs and glands, avoiding actual glandular structures, and the infrequency of metastasis, are features pointing toward chordoma rather than colloid cancer

5 Malignant chordoma may be considered as causing 100 per cent mortality. Because of the extensive infiltration of the regional fascias, and the difficulty of early diagnosis, operative treatment probably rarely effects a cure, but, of course, excisions of the principal tumor mass, and intestinal resections have been beneficial in the removal of pressure and pain phenomena.⁹

⁹ The following references also bear on this subject

Ewing *Neoplastic Disease*, 1919, p 188

Mallory *Principles of Pathologic Histology*, 1914, p 400

MacCallum *Textbook of Pathology*, 1918, p 1022

Adam *Principles of Pathology* 1 761, 1910

FURTHER OBSERVATIONS ON EXPERIMENTAL LESIONS OF THE BRANCHES OF THE AURICULOVENTRICULAR BUNDLE OF THE DOG

FRED M. SMITH, M.D.
CHICAGO

In some work¹ recently reported, lesions of the left branch of the auriculoventricular bundle were produced by incising the endocardium of the left ventricle. It was noted that dogs often gave a normal electrocardiogram even after extensive incisions had been made in the endocardium. Later, however, as the heart became dilated because of experimental ligation of coronary arteries the electrocardiograms were abnormal.

In this paper are recorded further observations that have been made following the division of the right and left branches and the subdivisions of the left branch of the auriculoventricular bundle. The extent to which the subdivisions of the left branch can be divided and the electrocardiogram still remain normal are in a measure determined. It is further shown that some of the atypical electrocardiograms may be brought back to normal.

In a series of thirty dogs - the right branch was divided in eight instances, the left in six and the subdivisions of the left branch in sixteen. The same method was used that was employed in the former work. Care was taken to divide the branches of the auriculoventricular bundle with a minimum damage to the cardiac musculature. We were successful in most instances in producing lesions of the conduction system with no other apparent disturbance of the function of the heart except that of the transmission of the impulse.

Lesions of the Right Branch of the Auriculoventricular Bundle — In eight dogs the main stem of the right branch was divided in the neighborhood of the region shown in Figure 1. The incision in six instances extended just through the endocardium. Following the incision there was an immediate change in the contraction of the heart.

* From the Medical Department of Rush Medical College and Presbyterian Hospital.

¹ This investigation was aided by funds given by Madam C. H. McCormick and R. T. Crane, Jr.

* Read before the Chicago Society of Internal Medicine, Dec. 20, 1920.

1. Smith, F. M. Experimental Observations on the Atypical QRS Waves of the Electrocardiogram of the Dog, Arch. Int. Med. **26**: 205 (Aug.) 1920.

2. These dogs were fully anesthetized.

that was recognizable by the naked eye. The ventricular chambers were apparently not contracting synchronously. This change in the contraction was so characteristic that we knew when the right branch was divided before taking the electrocardiogram. Other than the change in the contraction wave, the function of the heart was seemingly not disturbed in those instances in which the incision did not extend into the deeper layers of the musculature. The heart remained normal in size and appeared able to withstand an increased load as well as the normal heart.

Those dogs in which there was a complete division of the right branch of the auriculoventricular bundle gave a characteristic electro-



Fig 1 — Arrow points to the incision on the right side of the interventricular septum. This incision divided the right branch of the auriculoventricular bundle.

cardiogram (Fig 2). There was a marked S-wave in Leads II and III followed by a positive T deflection. The S-wave was usually notched or slurred at the apex and the duration of the QRS group was prolonged. The most prominent ventricular wave in Lead I was negative in five instances and positive in three.

The same type of electrocardiogram was obtained when a transient block of the right branch was produced by pressure. In one dog, slight pressure was made for a few seconds over the right branch. This caused a block of the impulse over this branch. The right coronary artery was then ligated and the right ventricle soon became

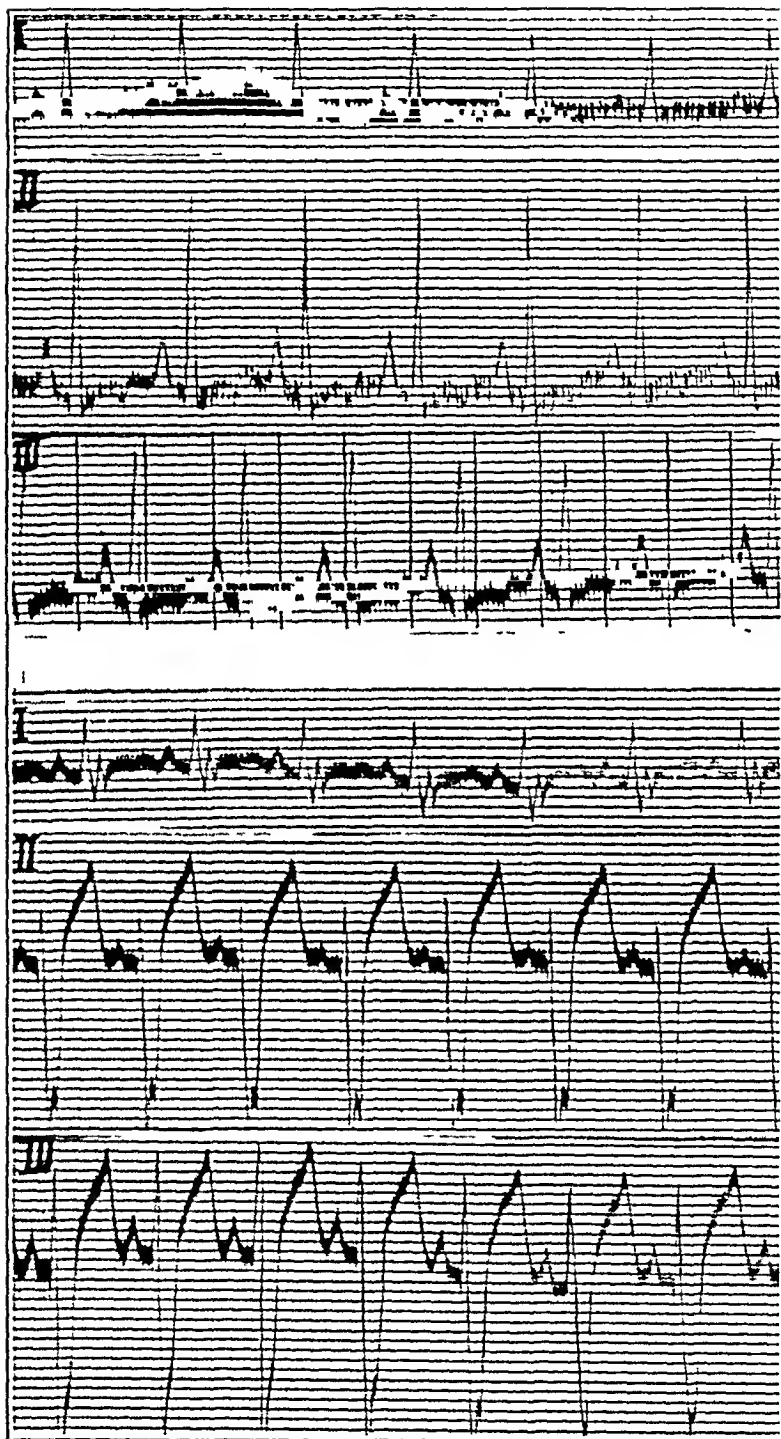


Fig 2—Top Normal electrocardiogram of the heart shown in Figure 1
Bottom Electrocardiogram taken after the right branch was divided

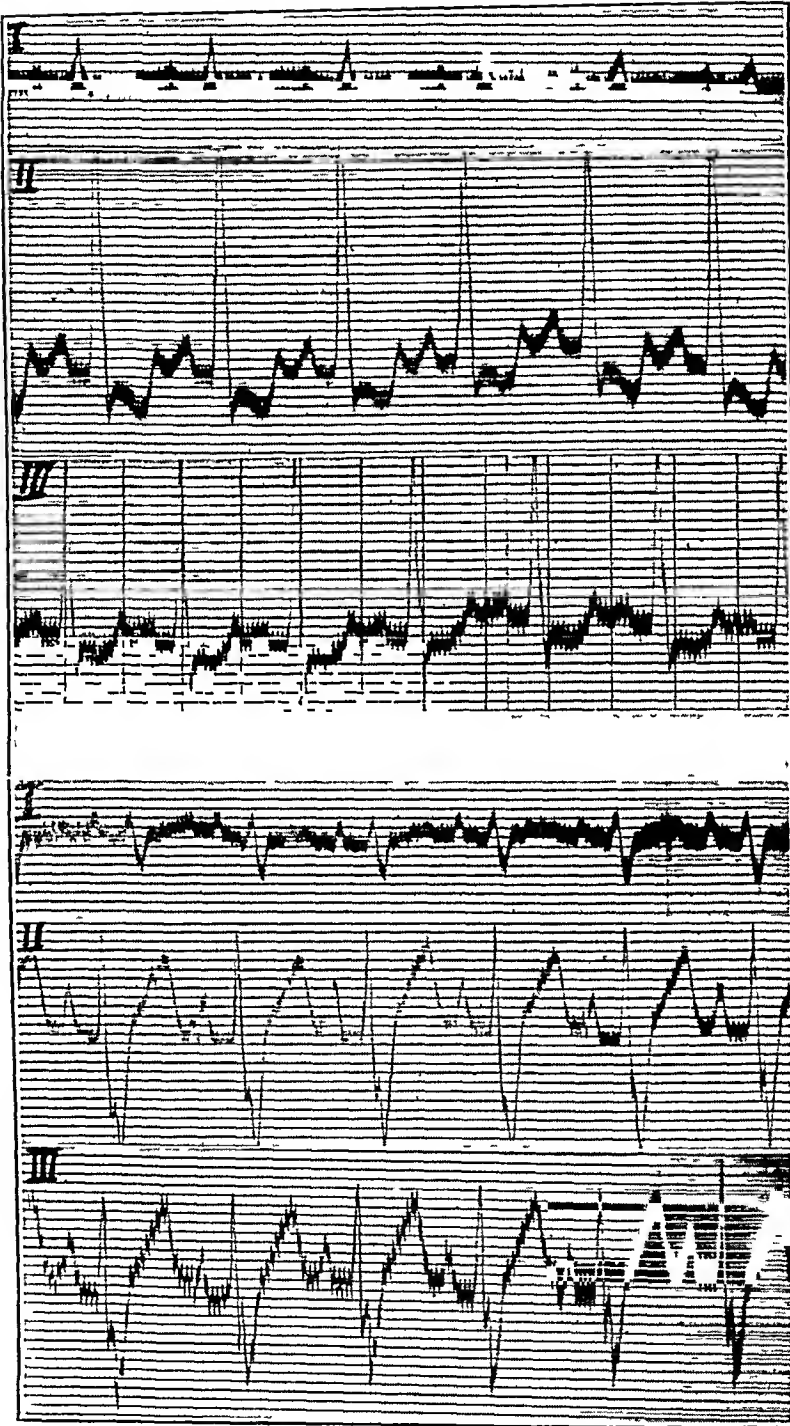


Fig 3—Top Normal electrocardiogram Bottom Record taken after pressure was made on the right branch

markedly dilated. The ligature was allowed to remain on the artery for one and one-half hours. During this time, a right bundle branch block persisted. As soon as the ligature was removed from the right coronary artery the right ventricle returned to the normal size and impulses began to pass over the right branch. Possibly, the temporary ligation of the right coronary delayed the return of the function of the right branch. In an experiment previously reported³ an electrocardiogram typical of that of right branch block appeared on the second day following the ligation of the right coronary artery.



Fig. 4—Shows several ragged incisions in the endocardium of the left ventricle below the point where the left branch breaks up into subdivisions. All the main subdivisions of the left branch were divided.

*Lesions of the Left Branch of the Auriculoventricular Bundle—*Because of the location of the left branch of the auriculoventricular bundle on the under surface of the septum, and its early breaking up into subdivisions, it was a difficult matter to divide the main stem of this branch with one incision. A small incision placed in the right position beneath the aortic valves would accomplish the desired results. It was, however, found to be a more certain procedure to depend on two or more small incisions just below the point where the main branch breaks up into subdivisions (Fig. 4).

³ Smith, F. M. Ligation of Coronary Arteries with Electrocardiographic Study, *Arch. Int. Med.* **22**: 8 (July) 1918.

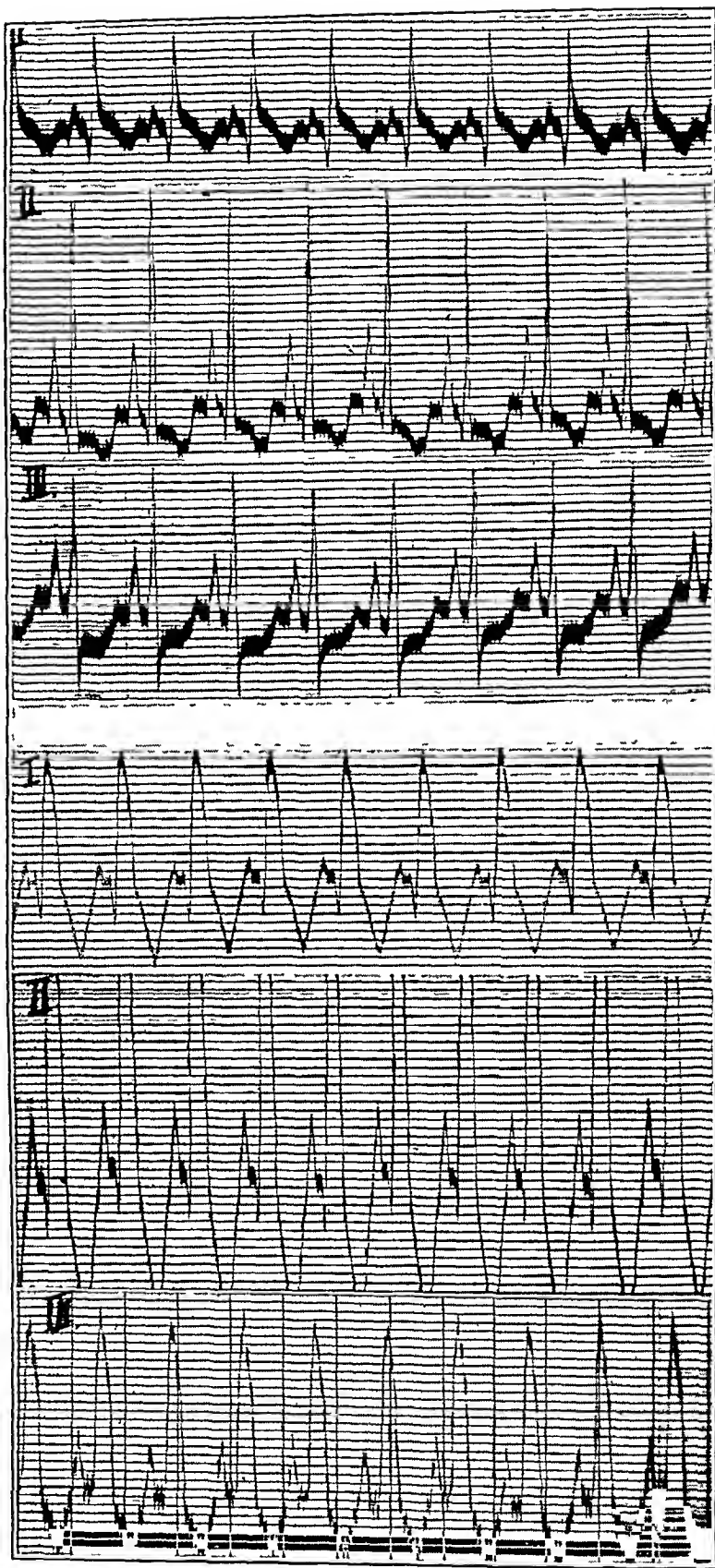


Fig 5—At the top is the initial electrocardiogram of the heart in Figure 4
The record at the bottom was taken after the incision was made in the
endocardium

In six dogs, the left branch was divided at the latter point. This was accomplished in four instances with but a small amount of damage to the ventricular musculature. As in the case of the division of the right branch, the section of the left branch could be determined by the change of the contraction wave of the ventricles as seen by the eye. This was always confirmed later by the electrocardiograms.

The electrocardiograms following the division of the left branch were of uniform type and as characteristic as those that followed the cutting of the right branch (Fig 5). The most prominent ventricular wave was upright in all leads, broad at the base and blunt and notched at the apex. The T-wave was a negative phase.



Fig 6—At the point marked X a small area of the endocardium was left intact.

The Division of the Subdivisions of the Left Branch of the Auriculoventricular Bundle—The former experiments¹ covered fairly well lesions of the subdivisions of the left branch. The present observations were made to determine the extent to which the subdivisions could be divided and the electrocardiogram remain normal. We also wished to see whether we could bring back to normal the atypical electrocardiograms produced by cutting the smaller subdivisions of the left branch and dilating the left ventricle by the ligation of the coronary arteries.

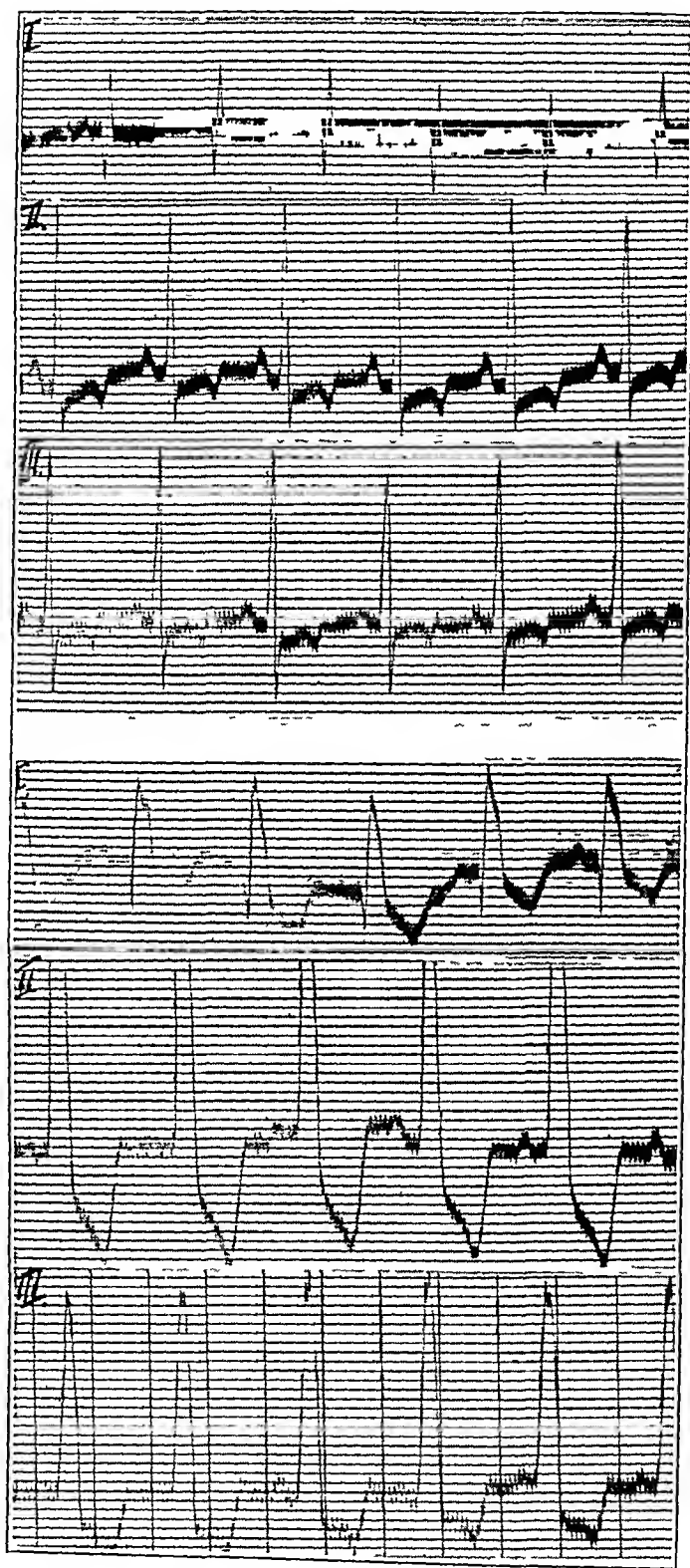


Fig 7—The upper figure is the initial electrocardiogram of the heart shown in Figure 6. The lower one was taken immediately after the incision was made in the endocardium.

In sixteen dogs, lesions of the subdivisions of the left branch were produced. The extent of the lesions varied from those involving the smaller ramifications to those involving two or more of the main subdivisions. In fourteen experiments, the incisions in the endocardium were very shallow and apparently did not disturb the muscular function of the heart. In two instances the incisions were more extensive than we anticipated. The heart soon dilated and the ventricles went into fibrillation.

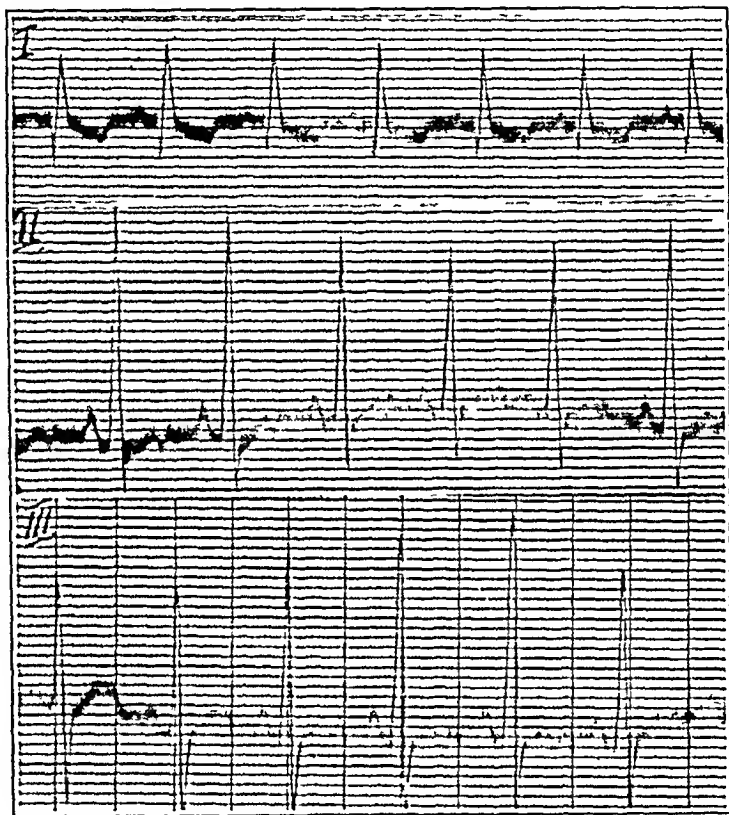


Fig 8—Electrocardiogram taken ten minutes following lower records in Figure 7

The division of the smaller ramifications and of the larger subdivisions of the left branch produced no change in the QRS group of the electrocardiogram. These results are in accord with those of my former work¹ and with those recently reported by Wilson and Herrmann⁴. The results in two experiments suggest the extent to which the main subdivisions can be divided and the electrocardiogram still remain normal. Immediately following the incision shown in Figure 6, the electrocardiogram was that of a complete branch block (Fig 7). Within ten minutes the electrocardiogram returned to normal (Fig 8).

⁴ Wilson, F. N., and Herrmann, G. R. Bundle Branch Block and Arborization Block, *Arch Int Med* 26:153 (Aug) 1920.

It is to be noted that the incision is not continuous (Fig 6) At the point marked X, a small area of endocardium was left intact One possible explanation of the result is that one of the main subdivisions may have passed between the incisions, and thus was not cut but was merely rendered temporarily functionless by the trauma of the knife In this way complete branch block resulted It also occurred to us that blood might have entered the connective tissue sheath surrounding the cut subdivision and produced a transient block of the remaining undivided subdivisions An electrocardiogram similar to the one shown in Figure 7 was obtained following the incision shown in Figure 9



Fig 9—Following the incision there was a transient block of the left branch There were at least two main divisions divided

Here, again, the electrocardiogram returned to the normal in a few minutes It would seem that at least two main subdivisions were divided by this incision

Return to Normal of Aberrant Electrocardiograms—In the work reported in 1920,¹ atypical electrocardiograms were produced by cutting subdivisions of the left branch and at the same time dilating the left ventric'e by the ligation of branches of the left coronary arteries No attempt was made in those experiments to bring these electrocardiograms back to normal In the investigations here reported, we attempted to see if these results could be secured In a measure, we were success-

ful Figure 10 shows an abnormal electrocardiogram that was produced by the above method, which returned to the normal after the ligatures were removed from the coronary arteries. This change in the electrocardiogram was produced four different times in one experiment. Figure 11 shows the incisions that were made in the endocardium.

It was not always possible to bring the heart back after the ventricles had been dilated to such a degree that the contraction was atypical. Yet we were successful in four experiments. In twelve dogs, the ventricles began fibrillating as soon as the ligatures were removed from the coronary artery. In six of these, the electrocardiogram did not become atypical until just before the onset of the ventricular fibrillation. The QRS group then resembled that of a premature ventricular contraction and the auricles were fibrillating.

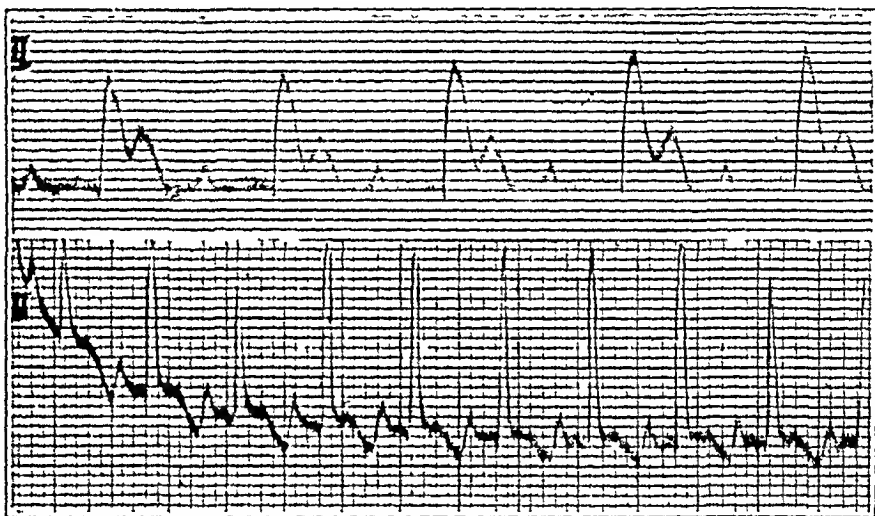


Fig 10—Top Lead II. Atypical electrocardiogram produced by dilatation of the left ventricle by ligation of the branches of the left coronary arteries following the incision in the endocardium shown in Figure 11. Bottom. The electrocardiogram returned to normal after the ligatures were removed from the coronary arteries.

This type of curve was due either to a complete bundle branch block or regular recurring ectopic ventricular contractions. Some of the electrocardiograms of the former work were of this type.

COMMENT

The results obtained in these experiments following the division of the right and left branches of the auriculoventricular bundle are in accord with those obtained by Eppinger and Rothberger,⁵ Lewis,⁶ and

⁵ Eppinger, H., and Rothberger, J. Ueber die Folgen der Durchschneidung des Tawaraschen Schenkel des Reizleitungs Systems, *Ztschr f klin Med* 70 1, 1910.

⁶ Lewis, T. *The Mechanism and Graphic Registration of the Heart Beat*. New York and London, 1920, p 117.

more recently by Wilson and Herrmann, and quoted by Eppinger and Stoerck. In the dog the diaphasic ventricular complex of large amplitude and increased duration in Leads II and III with the T-wave an exaggerated positive phase is diagnostic of right branch block. The aberrant positive wave in all leads followed by a negative T deflection is equally characteristic of a block of the left branch.

The criteria used in the diagnosis of right and left bundle block in the dog do not always seem to hold in man. In those few instances in which a careful histological examination of the conduction system has been made, the findings have at times been at variance with those which are expected from the electrocardiogram. Eppinger and Stoerck verified the electrocardiographic diagnosis of right branch



Fig 11—Shows incisions in the endocardium lateral and mesial to the posterior papillary muscle

block in two cases. Lewis⁶ mentions one case in which the anticipated lesion was found at necropsy. Cohn and Lewis,⁷ however, reported the histologic findings in four cases in which the electrocardiographic diagnoses were not substantiated. Oppenheimer and Pardee⁸ recently had the opportunity of studying two patients in whom the lesions were found in the branch opposite to that expected from the electrocardiogram.

7 Cohn, A. E., and Lewis, T. The Pathology of Bundle Branch Lesions of the Heart. *Proc New York Path Soc* 14: 207, 1914.

8 Oppenheimer, B. S., and Pardee, H. E. B. Site of the Cardiac Lesions in Two Instances of Intraventricular Heart Block. Read before the American Society of Clinical Investigation, Atlantic City, May 3, 1920.

The discrepancy between the anatomic and the electrocardiographic diagnosis may, in some instances, be due to the differences between the intraventricular conduction systems of man and the dog. This suggestion was offered by Oppenheimer and Pardee⁸ in explanation of the findings in their two cases. This, however, should hold only for lesions of the left branch, for, according to Lewis, it is only the right branch that differs materially in man and the dog. The electrocardiogram of right branch block in man should correspond fairly well with that produced experimentally in the dog. This was true in the cases reported by Eppinger and Stoerck and in the one mentioned by Lewis.⁶ It is to be remembered, too, that Cohn and Lewis,⁷ in commenting on the findings in their four cases, pointed out that functional block of either of the branches may exist. If, however, we assume that the electrocardiograms of right branch block in man and of the block produced experimentally in the dog are similar, it is very difficult to explain the findings in one of the cases reported by Oppenheimer and Pardee.⁸

Lewis,⁶ and Wilson and Herrmann⁴ have called attention to the similarity of the preponderance curves and those of bundle branch block. Those individuals in whom aberrant electrocardiograms are found have had in the majority of instances gradually progressive degenerative changes in the myocardium sufficient to disturb the muscle balance in the ventricles. It would seem that a preponderance of the left ventricle would not particularly modify the electrocardiogram of a right branch block in that individual. This, at least, is shown by Lewis⁶ by two curves from the same individual. The first was of right branch block. Later, the right branch functioned normally, and the electrocardiogram was that of left ventricular preponderance. The same might be expected of right ventricular preponderance and left branch block. When, however, the preponderance is on the same side as the branch block, one might expect that the electrocardiogram would be modified.

Lesions of the smaller subdivisions of the left intraventricular conduction system, even though they are extensive, do not necessarily change the form of the QRS group. This was noted in the former work,¹ and is in accord with the results of Wilson and Herrmann.⁴ In some instances, however, the electrocardiogram was changed to an atypical form by dilatation of the left ventricle caused by the ligation of branches of the left coronary artery. The fact that in four instances the curves returned to normal as soon as the ligatures were removed, and that this change in the form of the electrocardiogram was produced as many as four times in one of the experiments, would seem to indicate that functional changes in the myocardium may influence the transmission of the impulse within the ventricles.

The division of one or more of the main subdivisions also did not change the form of the Q R S group. It would seem from two of these experiments that the impulse may reach all parts of the left ventricle in normal time if one main branch is left intact. This is only possible through the free anatomic arborization between the main branches.

SUMMARY

Complete bundle branch block in the dog produces characteristic electrocardiograms that are diagnostic of the lesion. The division of the smaller subdivisions or even one or more of the main subdivisions did not change the form of the Q R S group. In some instances, however, these waves were changed to the atypical form after the cutting of subdivisions, by dilatation of the left ventricle, produced by ligating branches of the coronary arteries. This would indicate that functional change in the myocardium may influence the conduction of the impulse within the ventricles.

PRIMARY HEMANGIOLYMPHOMA OF THE HEMAL NODES AN UNUSUAL VARIETY OF MALIGNANT TUMOR

DOUGLAS SYMMERS, MD

NEW YORK

In man and in certain of the lower vertebrates, nodal collections of lymphoid cells occur, and are divisible into two groups, namely, those which are provided with lymph sinuses, or ordinary lymph nodes, this group comprising by far the larger number, and those which are provided with blood sinuses, the latter being variously known as hemolymph nodes splenolymph nodes, or more simply and better, perhaps, as hemal nodes. The exact anatomic position of the hemal nodes has not been determined. The preponderance of evidence, however, favors the view originally advanced by Vincent and Harrison,¹ that they are more closely related to the spleen, structurally and otherwise, than to the lymph nodes proper. In fact, Meyer,² in a series of injection experiments in sheep, has shown that the hemal nodes are in no way connected with the lymphatic system. Embryologically, the hemal nodes, like the spleen, are vascular bodies or masses of erectile tissue that have become modified in the process of development by the interposition of focal collections of lymphocytes in the meshes of a reticulum derived from the walls of blood vessels.³

In certain animals, notably in the bullock and sheep, hemal nodes are normally present and are conspicuous by their reddish color. In man, on the contrary, the hemal nodes in health, although present, are seldom, if ever, distinguishable by the naked eye, but in certain conditions of disease, particularly in anemias associated with changes in the spleen and bone marrow, and in syphilitic lesions of the spleen attended by overgrowth of connective tissue, they frequently come into view in the prevertebral fat tissues of the neck and thorax, and in the abdomen, more especially in the region of the promontory of the sacrum, as reddish or brownish red, rounded, oval or almond shaped bodies, varying in size from a few millimeters to 1 or 2 cm. Confirmatory evidence of their relationship to the spleen is furnished by Tizzoni⁴ who, after experimental splenectomy in the dog, noted the occurrence of minute reddish nodules in the abdominal fat that he

1 Vincent and Harrison. *J. Anat. & Physiol.* **11** 176, 1897

2 Meyer. *Anat. Rec.* **2** 62, 1908

3 Sabin. *Keibel & Mall's Human Embryology*, **2** 750, 1912

4 Tizzoni. Quoted by Mosler

examined minutely and interpreted as collections of newly formed splenic tissue. Similar results have been obtained by Winogradow,⁵ Warthin,⁶ and others, the latter designating the bodies in question as hemolymph nodes, although recognizing their close structural resemblance to the spleen and their apparent similarity of function.

In certain lower forms of life, the spleen is a divided organ, and is more or less widely distributed through the abdomen, whereas in higher animals it usually occurs as a compact body. For example, in some of the invertebrate fish the spleen is strewn through the subserous tissues of the gastro-intestinal tract,⁷ while in slightly higher forms it becomes separated and distributed in the tissues that run parallel with the stomach and intestine. In still higher fish, particularly among the selacians, the spleen consists of more or less completely separated nodules, a fact which tends to explain the occurrence of accessory spleens in man, in whom they are commonly encountered, from one or two to a dozen or more. In the case of an adult male, reported by Albrecht,⁸ there was no evidence of a common spleen. On the contrary, splenunculi were scattered through the peritoneum to the extent of 400 or more. These splenunculi were highly vascularized, standing out as reddish or brownish red bodies which, at first glance, suggested the presence of a metastasizing tumor and, microscopically, were found to be plentifully supplied with lymphoid cells, the naked eye and histological appearances thus differing in no essential particular from the hemal nodes. Taking all things into consideration, it would seem that the hemal nodes represent an accessory system of diminutive spleens, whose prototype is to be found in the auxiliary system of lymph nodes occurring in man in the form of minute foci of lymphoid cells lying in the interstitial tissues of the thyroid, prostate, testicle, lungs, kidneys and adrenals, in the subcutaneous and submucous tissues and serous membranes, and elsewhere.⁹ These deposits, like the hemal nodes in health, are small, and may escape detection in the routine histologic examination of tissues. Like the hemal nodes, again, they may be brought into prominence in disease, especially in conditions characterized by lymphoid hyperplasia, such as Hodgkin's disease, lymphosarcoma, pseudoleukemia, and in enteric fever.

As far as I have been able to learn, tumors in human beings springing from the hemal nodes have not hitherto been described. Of peculiar interest in this connection, however, is the observation of

⁵ Winogradow. *Centralbl f d med Wissensch* **50** 900, 1882

⁶ Warthin. *J M Research*, **2** 435, 1902

⁷ Sobotta. *Anatomie der Milz*, Jena, 1914, p 283

⁸ Albrecht. *Betr z path anat u z allg Path* **20** 513, 1896

⁹ Symmers. *Arch Int Med* **21** 237 (Feb) 1918

Mosler¹⁰ who, in a dog, ten months after splenectomy, noted the occurrence of innumerable small, circumscribed, reddish nodules scattered through the omentum and mesentery, that closely resembled the structures identified by Tizzoni in animals similarly treated as newly formed masses of splenic tissue. In Mosler's animal the histologic examination was made by Roth, who interpreted the reddish bodies as neoplastic formations and termed them hemorrhagic telangiectatic lymphomata.

In the pathological laboratories at Bellevue Hospital, I have had occasion to study two malignant tumors composed of groups of lymphoid cells associated with vast numbers of blood channels, the structure of the growths, as a whole, presenting a histologic picture that suggests derivation from the hemal nodes. One of the tumors was encountered as an accidental finding at necropsy in an adult male who had met death as a result of cyanid poisoning. The growth lay in the concavity of the left side of the pelvis and was about the size of an apple and reddish gray in color, while in the immediate vicinity and extending upward to the level of the first lumbar vertebra were a score or more of enlarged nodes of the same general description. The second case was that of a child, 3 years of age, and was marked by a huge collection of reddish tumor masses in the abdomen and by an extraordinary display of metastases distributed exclusively under the periosteum of various bones.

REPORT OF CASES

CASE 1.—Nothing is known of the patient's clinical history. The body was subjected to necropsy in the pathological laboratories of Bellevue Hospital under the authority of the chief medical examiner of the City of New York, Dr. Charles Norris, to whom I am indebted for permission to present the findings.

Necropsy 7209—The body was that of a well nourished man, 28 years of age. The general configuration was that of status lymphaticus. On section, a thymus weighing 20 gm. was found in the usual situation. On opening the abdomen, a tumor came into view lying in the concavity of the pelvis on the left side of the sacrum, reaching just to the brim of the pelvis. The mass measured 7 by 5 by 4 cm. and was rounded, deep reddish gray in color, homogeneous in appearance and rather soft in consistence. On section, the growth presented a perfectly smooth, bluish red surface in which innumerable minute reddish specks could be seen alternating with faintly grayish areas. In the immediate vicinity of the tumor were ten or a dozen rounded or oval nodules, measuring from 2 to 3 cm. in diameter. On section, they presented essentially the same appearance as that of the larger growth. A chain of similarly enlarged lymph nodes extended upward on the left side of the aorta from the region of the pelvic growth to a point corresponding to the body of the first lumbar vertebra. These nodes were rounded or oval in outline, discrete, reddish gray in color and, on section, showed the same finely speckled appearance. The rest of the organs revealed no naked eye or histological changes worthy of note in the present connection.

Histologic Examination—Sections were removed from the main tumor mass and from several of the enlarged lymph nodes in the vicinity, and were stained

¹⁰ Mosler. *Deutsch. med. Wchnschr.* 22: 337, 1884.

with hematoxylin and eosin and by the method of Van Gieson. Histologically, the growths were characterized by a great profusion of small, thin walled blood vessels, the lumina of which were distended by closely packed red blood corpuscles. Scattered between the vascular channels were diffuse collections of small round cells corresponding in all particulars to lymphoid cells as familiarly encountered. In other places rounded or oval lymphocytic foci occurred, the cells of which were rather loosely packed and arranged around small blood vessels whose walls, in many instances, were thickened and hyalinized, staining reddish by Van Gieson's method, the formation as a whole, presenting a distinct resemblance to the malpighian bodies of the spleen. At the extreme

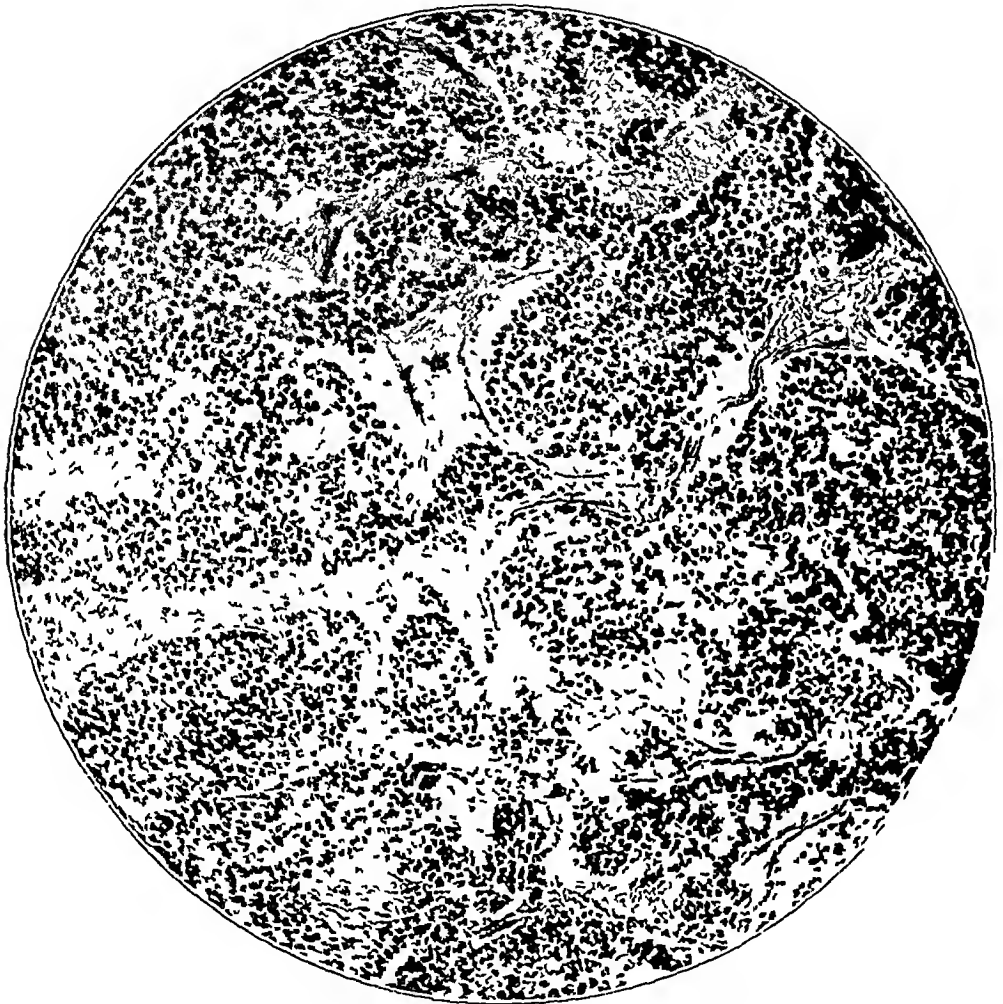


Fig 1, Case 2—Low power photomicrograph showing general topography of the tumor

periphery, the tumor was limited by a rather thick connective tissue capsule which occasionally sent prolongations downward for a short distance, but, as a whole, trabeculation of the deeper parts of the growth was entirely missing and, in fact, with the exception of an occasional delicate strand of connective tissue, as revealed by Van Gieson's stain, no stroma was apparent, the lymphocytic cells being supported partly by reciprocal pressure and partly by the abundant network of small blood vessels around and between which they were distributed.

CASE 2—The patient, a female child, 3 years of age, was admitted to Bellevue Hospital May 5, and died four months later. It was not possible to obtain a satisfactory clinical history. The child is said to have felt indisposed for a

period of only three weeks before admission to the hospital, at which time small lumps appeared in the inguinal and femoral regions. The patient gradually became pale and weak and a tumor appeared above the right eye, the tissues in the immediate vicinity presenting a bluish discoloration. Physical examination showed an anemic poorly nourished child. Above and to the outer side of the right eye was a firm tumor, about the size of a small walnut. The skin covering the tumor and that of the corresponding eyelids was slightly discolored, and the veins above the growth were engorged. Over the posterior parietal and occipital regions were several other masses of the same consistence, but somewhat smaller in size. In the abdomen a large, irregular, firm growth was

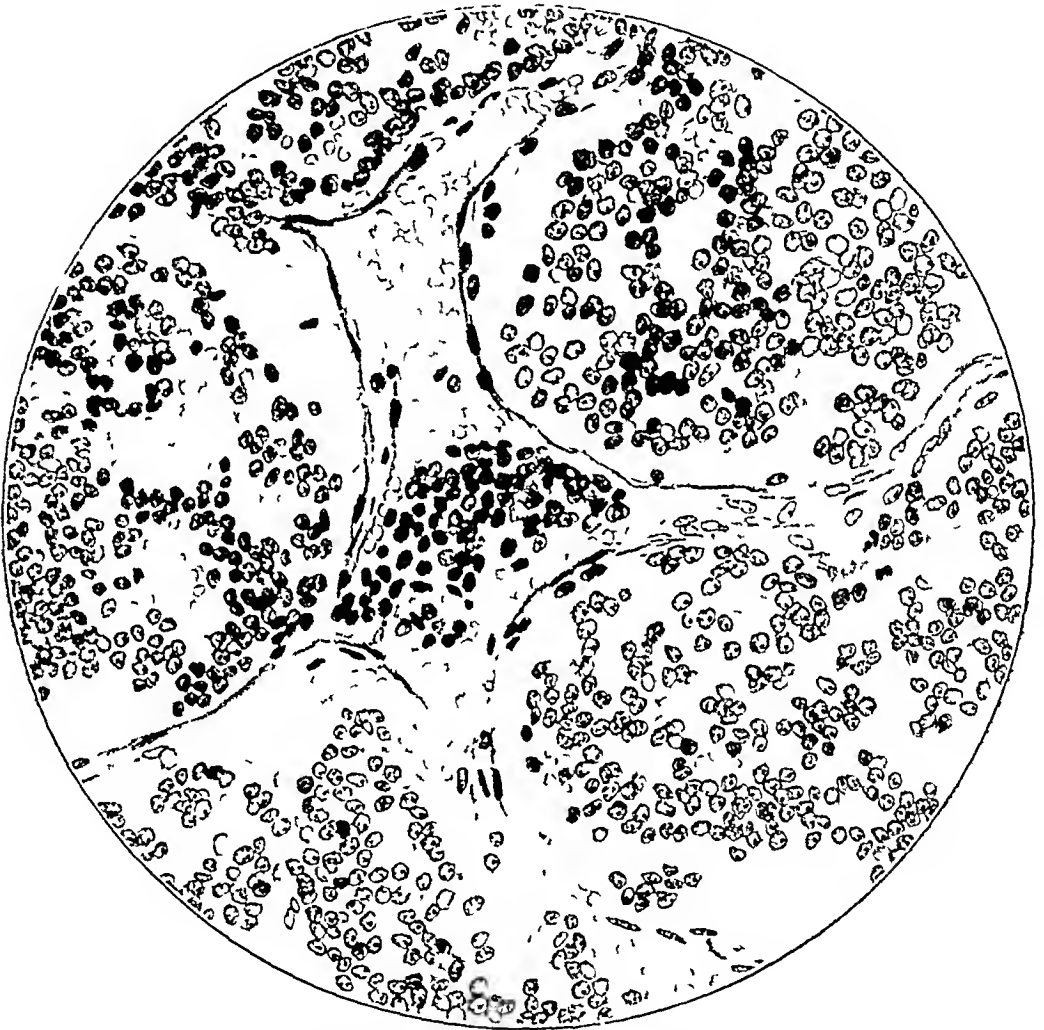


Fig 2, Case 2—High power drawing made from field corresponding to the center of the photomicrograph shown in Figure 1. The drawing illustrates the vascular channels lying in the supporting connective tissue, the presence of tumor cells in them, and free red corpuscles lying in the alveoli of tumor cells.

felt on the left side, extending from the level of the costal slope downward as far as the crest of the ilium and inward to the median line. White blood cells, 9,000, red cells, 1,500,000, hemoglobin, 30 per cent.

Necropsy 3766—The body was that of a child, 3 years of age, weighing 27 pounds and measuring 97 cm in length. The skin was pale. The arms and legs were thin, the abdomen prominent. The right upper eyelid was dark bluish in color, the lower eyelid faintly greenish. The conjunctivae were pale, but otherwise normal in appearance. On opening the abdomen, a small amount

of slightly blood tinged fluid was present in the pelvis and in the dependent portions of the peritoneal cavity. In the left hypochondrium was a massive tumor covered by peritoneum. The growth was globular in shape, measuring 10 x 9 x 6 cm. On section, it was found to be made up of a series of fused lymph nodes which were bright red in color. In the center of the mass was an area of liquefaction necrosis. The retroperitoneal lymph nodes were greatly enlarged and, on section, were bright reddish in color with alternating areas of whitish appearance and, together with the large globular tumor mentioned weighed 650 gm. Enlarged lymph nodes of the same reddish color were present in the iliac region, in the anterior mediastinum, around the roots of the bronchi, and along the sides of the esophagus, some discrete, others fused. In the lower and external portion of the right frontal bone, near the orbit, was a tumor that measured 3 x 1 cm. On removing the scalp, which was edematous and somewhat thickened, it was found that the calvarium, as a whole, presented an irregularly mottled appearance, due to the presence of numbers of deep bluish foci, varying in size from $\frac{1}{2}$ to 2 cm, corresponding to the distribution of tumor tissue beneath the periosteum. Large tumor nodules were revealed near the junction of the parietal and occipital bones, close to the sagittal suture, and a series of bluish foci, the whole measuring 4 or 5 cm in length, was situated along the coronal suture near the longitudinal fissure. Near the posterior and median part of one of the parietal bones, was a mass that measured 2 by $2\frac{1}{2}$ cm and which seen through the epicranium, presented a deep bluish color. On the external surface of the right occipital bone was still another bluish tumor mass which approximated 2 cm in diameter. Over the whole extent of the inner surface of the calvarium and extending well down the sides of the vault of the cranium, the dura mater was dark bluish in color and the interval between it and the bone was extensively occupied by reddish, soft tumor tissue. On peeling the dura from the calvarium, some tumor tissue remained adherent to the bone and some to the dura. In places the bone was superficially infiltrated, presenting a finely roughened appearance, as if punched out with small pins. There was a smaller amount of tumor tissue of the same character on the base of the skull, corresponding to the petrous portion of the right temporal bone and to the processes and body of the sphenoid and the clivus. The sternum in its middle portion anteriorly presented a bluish appearance which, on section, was found to correspond to reddish tumor tissue lying immediately under the periosteum. The inner surface of several of the ribs was finely speckled with bluish areas, pinpoint to pinhead in size, or slightly larger. In the lowermost ribs on both sides were large, deep bluish areas which, on section, corresponded to the presence of reddish tumor tissue lying between the periosteum and bone. In places, the surface of the rib appeared to be superficially infiltrated and softened by the growth. The largest bluish masses were found near the costal cartilages. Below the head of the right humerus there was a considerable sweep of infiltrating tumor tissue lying immediately under the periosteum. The lower half of the right femur showed large masses of tumor tissue beneath the periosteum, just above the right condyle. Where the tumor was most abundant, the cortex of the shaft was thinned and infiltrated. Section of the lower half of the right femur failed to show the presence of tumor tissue in the marrow. The periosteum of the anterior and left lateral surfaces of the sixth and seventh dorsal vertebrae was bluish in color and on section, was found to be infiltrated by reddish tumor tissue which brought about superficial erosion of the bone. Section through the entire anterior half of the bodies of the vertebral column failed to show tumor tissue within the marrow. On removing the spinal cord, it was found that there were small areas of neoplastic infiltration beneath one or two of the laminae and under the posterior surface of the body of one of the lower dorsal vertebrae.

The thymus was small, symmetrical in outline, and weighed approximately 10 gm. The gastro-intestinal tract, lungs heart liver spleen suprarenal

capsules, kidneys and other viscera showed no changes worthy of record in the present connection

Histologic Examination—Microscopic examination of sections removed from the primary growth in the abdomen showed the presence of great numbers of richly chromatic, rounded cells arranged in alveoli and surrounded by a highly vascularized connective tissue stroma. The tumor cells were rather loosely packed and the alveoli varied in shape and size. The individual tumor cells were somewhat larger than the ordinary lymphocyte and were provided with a rounded, fairly deeply chromatic, smooth or slightly granular nucleus, and very little cytoplasm. The connective tissue stroma was arranged in the form of delicate fibrils which frequently separated in such fashion as to surround sinuses of different shapes and sizes—some small and slitlike, others rounded, oval or angulated, practically all of them filled or distended by red cells. In many instances it was apparent that rupture had occurred, in which circumstances larger or smaller numbers of free red corpuscles were to be found strewn among the tumor cells or arranged in clumps. Massive hemorrhages were not uncommon. In many of the alveoli delicate, stringlike structures were to be seen insinuating themselves between the tumor cells or lying in definite bundles, representing, apparently, fibrin or some related substance. Areas of necrosis were numerous and often large. In one of the sections removed from the globular tumor in the abdomen, a few of the alveoli showed the presence of relatively huge, rounded or polygonal cells with abundant pale, finely granular cytoplasm and a centrally or somewhat eccentrically placed vesicular or fairly deeply staining nucleus. Cells of this type were encountered in the section in question to the number of only about twenty and, as a rule, they were caught in the meshes of the pinkish, thread-like masses of fibrin or fibrinoid material. Similar cells were not seen in any of the metastatic foci that were examined microscopically, and I am at a loss to account for them unless they represented tumor cells undergoing regressive changes, perhaps as a result of autolysis. Otherwise the microscopic examination of the metastatic deposits revealed an histology not to be distinguished from that of the original growth, that is to say, groups of lymphoid cells separated from one another by a stroma provided with blood sinuses.

CONCLUSIONS

- 1 There is a form of malignant tumor belonging to the lymphocytic group in which the naked eye and histological characteristics are such as to indicate derivation from the so-called hemal nodes, that is to say, the tumor arises in that locality where the hemal nodes are most frequently encountered, has a distinctive reddish or bluish red color and, microscopically, more or less faithfully counterfeits the histology of the hemal nodes in that it is composed of groups of lymphoid cells separated by innumerable distended blood sinuses with or without a supporting reticulum of connective tissue.

- 2 Two forms of neoplastic growth are described in this paper—one occurring as a solitary mass without metastases, but attended by secondary nodules arising regionally, and hence to be classified as locally malignant; the other giving rise to multiple selective subperitoneal metastases, and hence highly malignant.

3 The variety of tumor described is apparently satisfactorily provided for under the appellation of malignant hemangiolymphoma or as hemangiolymphosarcoma

4 Experience in the postmortem rooms of several hospitals has left me with the impression that primary tumors of the sort referred to in this paper occur with a fair degree of frequency I do not refer to tumors of this type with multiple selective subperiosteal metastases—growths of this distribution, I assume, are pathologic curiosities—rather to tumors which present in a general way the ordinary characteristics of lymphosarcomata or malignant lymphomata, but which differ from them in that they are of a striking reddish or bluish red color I have examined the only postmortem records at present available to me, namely, those of Bellevue Hospital, comprising some 7,000 protocols, but have not been able to find more than the two examples recorded in this paper Once attention is directed to the existence of primary tumors of this sort, however, it should serve to attract observations from independent sources and thus to clarify our knowledge of the incidence and behavior of the highly vascularized neoplasms of the lymphocytic family

5 The preponderance of evidence seems to favor the view originally advanced by Vincent and Harrison, that the hemal nodes are more closely related to the spleen, structurally and otherwise, than to the lymph nodes proper—that they represent a succession of diminutive spleens corresponding to the auxiliary lymphomatous deposits that normally exist in the interstitial tissues of many organs

THE CHEMISTRY AND CLINICAL SIGNIFICANCE OF UROBILIN*

LOUIS BAUMAN, M D
NEW YORK

Renewed interest in the physiology and clinical significance of urobilin has been aroused by the work of Wilbur and Addis¹ in this country, and by that of H Fischer² and collaborators abroad. Clinical reports by Schneider,⁴ Howard and Hansmann,⁵ McCrudden,⁶ Robertson,⁷ Giffin, Stanford and Szlapka,⁸ have served further to illustrate the practical value of urobilin determinations, in the bile, urine and feces.

In the present article, I wish briefly to review the present status of the urobilin question, and to tabulate the results of about 100 determinations on the urine and stool.

If we could determine accurately the daily excretion of bilirubin, it might aid in gaining an insight into this particular function of the liver and also afford a clue as to the extent of blood destruction, for at present it is generally believed that bilirubin is directly derived from hematin. Unless the patient or experimental animal has a biliary fistula, it is not possible to determine the bilirubin production, and even then we are assuming that the analytical methods at our disposal are sufficiently accurate to do so. If we attempt to estimate the bile pigments in the stool, we meet with at least two disturbing factors, the one is the difficulty of accurately separating the twenty-four hour amount of stool, and the other is the unknown factor of intestinal absorption and destruction. The errors arising from these uncertainties may be diminished somewhat by determining the average excretion for three or more days.

The normal stool, however, does not contain bilirubin itself, but instead two derivatives are found, which are closely related chemically, yet totally different in certain other respects. These substances are known as urobilin and urobilinogen respectively.

*From the Medical Clinic, Presbyterian Hospital, Columbia University

1 Wilbur, R L, and Addis, T. *Arch Int Med* **13** 235 (Feb) 1914

2 Fischer, H. *Ztschr f Phys Chem* **73** 204, 1911

3 Fischer, H, and Meyer, P. *Ztschr f Phys Chem* **75** 339, 1911

4 Schneider, J P. *Arch Int Med* **17** 32 (Jan) 1916, *J A M A* **74** 1759 (June 26) 1920

5 Hansmann, G H, and Howard, C P. *J A M A* **73** 1262 (Oct 25) 1919

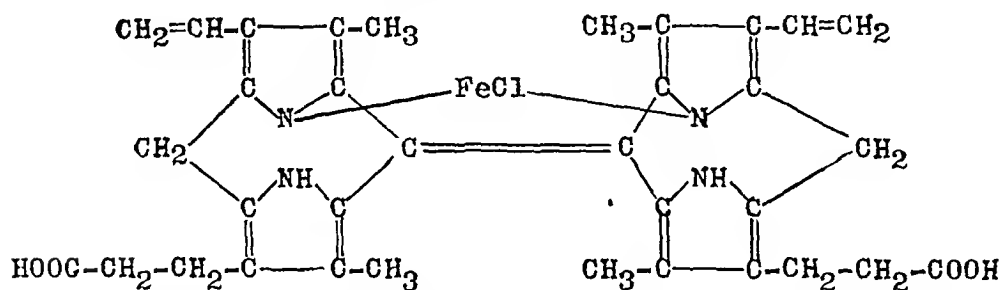
6 McCrudden, F H. *Boston M & S J* **177** 907, 1917

7 Robertson, O H. *Arch Int Med* **16** 472 (Sept) 1915, *Arch Int Med* **16** 549 (Oct) 1915

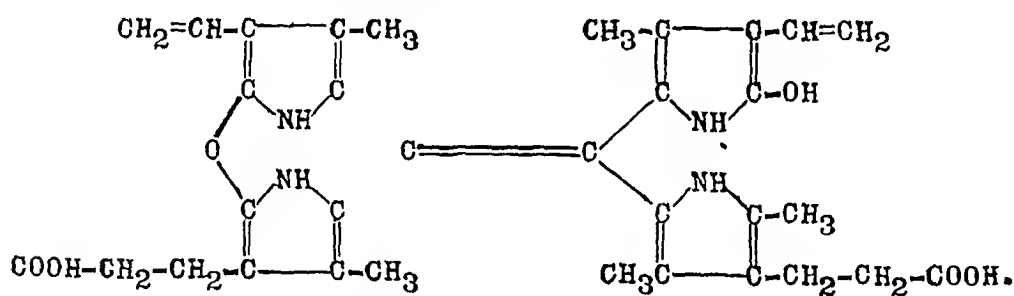
8 Giffin, H Z, Stanford, A H, and Szlapka, T L. *Am J M Sc* **155** 562, 1918

In 1868, Jaffe ⁹ described a reddish brown substance which he found in human and canine bile and which resembled one of the pathologic urine pigments. He found that both compounds absorbed certain rays between the *B* and *F* lines of the spectrum, and both exhibited fluorescence in the presence of zinc salts. The new pigment was called urobilin. It is remarkable that even at that early date, Jaffe was aware that the pigment resulted from the oxidation of a colorless chromogen which is now known as urobilinogen.

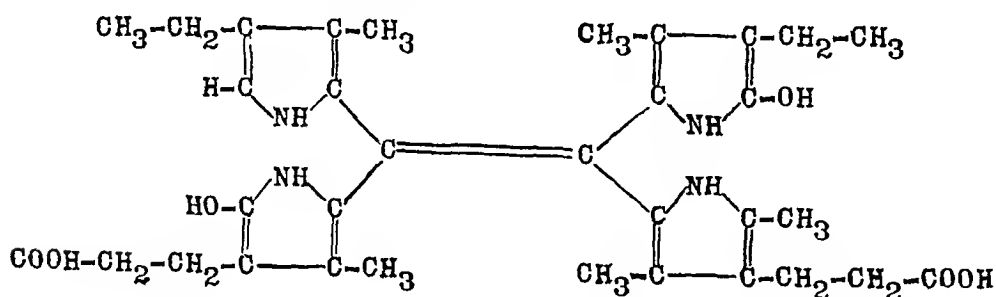
Hemin



Bilirubin



Urobilinogen



Urobilinogen has the formula $\text{C}_{33}\text{H}_{42}\text{O}_6\text{N}_4$. Fischer and Roese ¹⁰ demonstrated that in common with hemin and bilirubin, it has four pyrole nuclei.

The formulas show that the three compounds are closely related, for in addition to the pyrole nuclei, each compound contains eight side

⁹ Jaffe M. Centralbl f d med Wissensch 243, 1868, Centralbl f d med Wissensch 177, 1869

¹⁰ Fischer, H. and Roese, H. Ztschr f Phys Chem 89 25, 1914

chains which are almost identical in the three substances. Bilirubin may be regarded as an oxidized hemin while urobilinogen is a reduced bilirubin. This close chemical relationship cannot be ignored in a discussion of the derivation of the bile pigments, it is surely a weighty argument, favoring the view that bilirubin is directly derived from hematin.

Urobilinogen is a colorless crystalline compound which is quite soluble in many of the organic solvents. In the presence of oxygen or oxidizing substances, it is readily converted into urobilin. The close chemical relationship between bilirubin and urobilinogen is further illustrated by the ease with which the latter can be synthesized from the former substance. H. Fischer³ was able to obtain urobilinogen in a 50 per cent yield by reduction of bilirubin with sodium amalgam. He assumed that it was formed from one-half of the bilirubin molecule and accordingly named it hemibilirubin. Later he and Meyer-Betz¹¹ proved that urobilinogen (obtained from pathologic urine), and hemibilirubin were identical.

When urobilinogen is treated with Ehrlich's reagent, that is a solution of para dimethylaminobenzaldehyde in hydrochloric acid, a reddish condensation compound is formed which absorbs rays in the orange and green regions of the spectrum between the *D* and *E* lines. This red substance is strongly colored, one part of urobilinogen in 640,000 parts of water being sufficient to give the reaction,³ and it is, therefore, well adapted for its determination. The reaction is not specific, for it may be obtained with any pyrole derivative that has a free hydrogen atom joined to one of the carbon atoms of the ring.

Urobilin is a reddish brown substance of uncertain composition. It is apparently a conglomerate of urobilinogen molecules that have been oxidized and polymerized. It is soluble in most of the organic solvents, is resinous in nature and exhibits fluorescence in the presence of zinc salts. It absorbs certain rays of the spectrum in the neighborhood of the *B* and *F* lines. With mercuric chloride, it forms a reddish compound, the basis of the so-called Schmidt test. Urobilin can be reduced to urobilinogen in vitro by bacteria.

OCCURRENCE

Because urobilinogen and urobilin have the same clinical and physiologic significance, and for the sake of brevity, the term urobilin will be used to include both substances. Urobilin occurs in negligible quantities in the urine of normal people. Little definite information is available concerning its presence in the blood. We have attempted

¹¹ Fischer, H., and Meyer-Betz, F. *Ztschr f Phys Chem* **75** 232, 1911, Fischer, H., and Meyer-Betz, F. *Muench med Wchnschr* **59** 799, 1912

to demonstrate it in the serums of patients who were excreting considerable quantities in the urine, but without success. When normal serum is heated with strong hydrochloric acid, it gives a positive Ehrlich reaction, but this may well be due to the liberation of tryptophane or its decomposition products. Some investigators claim to have found urobilin in the serum of pneumonia patients shortly before death. When it is added to blood, it disappears rapidly. Increased amounts of urobilin have been found in the stool and urine in diffuse hepatic lesions and in pernicious anemia and other conditions associated with a rapid and increased destruction of red blood cells. It is absent or almost absent from the stool in jaundice due to complete closure of the common bile duct and in severe diarrhoeas.

MECHANISM OF UROBILIN FORMATION

The voluminous literature dealing with this phase of the subject abounds in lengthy theoretical discussions and hypotheses. The so-called enterogenous theory had its chief exponent in Friedrich Mueller.¹² It appears to be least open to criticism and is best supported by experimental observations. In brief, it postulates that urobilin results from the reduction of bilirubin by the bacteria of the large intestine. The following evidence is submitted in support of the enterogenous theory: (1) The transformation of bilirubin into urobilin by bacteria *in vitro*. (2) The absence of urobilin in the stool in severe jaundice due to obstruction of the common bile duct. If, to such patients, bile be administered (by stomach tube) urobilin promptly appears in the stool. (3) Although bilirubin occurs in the stool of the new-born, urobilin is not present until the third day, when the bacterial flora of the intestine have had an opportunity to develop. (4) Diarrheal stools contain bilirubin but often no traces of urobilin. It is believed that increased peristalsis produces a rapid passage of bile through the intestine before the bacteria have had time to reduce bilirubin to urobilin.

Normally, some urobilin is absorbed from the intestine and brought to the liver where it is probably converted into bilirubin. If a large amount is absorbed, or if the liver is extensively diseased, then it may escape into the general circulation and be excreted by the kidneys. In pernicious anemia and hemolytic jaundice or as a result of the action of certain poisons, a large number of red cells are destroyed per unit of time, and the liberated hematin is changed into bilirubin, which, in turn, leads to an increased amount of urobilin in the stool. In recent years, hematin and bilirubin have been demonstrated in the blood serum in these pathologic conditions.

¹² Mueller, F. *Ztschr f klin Med* **12** 45, 1887, *Kongr f inn Med* **11** 118, 1892.

The method which is now commonly used for determining urobilin is that of Wilbur and Addis¹. A portion of the urine or fecal extract is treated with alcoholic zinc acetate solution and with Erlich's reagent. The mixture is then diluted until the spectral absorption bands of urobilin and the urobilinogen compound just disappear. The average normal excretion in the twenty-four hour stool, according to Wilbur and Addis, is about 6,500 dilutions. In making the calculations for the stool, we purposely have omitted to consider the initial dilution due to admixture of reagents in order to make our results comparable with those of Wilbur and Addis.

CLINICAL SIGNIFICANCE

An increased amount of urobilin in the urine is frequently observed in diffuse lesions of the liver, as in fatty and parenchymatous degeneration, cirrhosis, diffuse neoplasm and quite constantly in the congestion of heart disease. Urobilinuria is found in the liver degeneration due to the acute infectious diseases, especially scarlet fever, rheumatism and lobar pneumonia.

When the flow of bile to the intestine is interrupted, the amount of urobilin in the stool is diminished, or it may be entirely absent, depending on the degree of obstruction of the bile duct. When bile or urobilinogen itself is administered to normal subjects, there is no increase of urobilin in the urine, but when administered to patients with liver disease, considerable quantities may appear therein.

In the various hepatopathies, we have usually found an increase in urobilin, either in the stool alone or in the urine alone, or in both.

An increased amount of urobilin in the stool is observed in cases of hemolytic jaundice and pernicious anemia, while in secondary anemia, a normal or subnormal excretion is the rule. Urobilin estimations afford a valuable means of differentiating the two conditions unless the liver itself is the seat of widespread pathologic change. Schneider⁴ observed a marked increase of urobilin in the bile of pernicious anemia patients and his observations have been confirmed by investigators at the Mayo clinic.⁸ We have examined the feces, the bile and the urine and have found the stool examination to be the most satisfactory. Attempts to demarcate the twenty-four hour amount of stool have been futile. We have observed large quantities of urobilin in the stool of pernicious anemia patients when microscopic examination of the blood showed no abnormality. Occasionally, the urobilin content of the stool decreases during the remission so frequently observed in this disease. While the method is far from accurate, especially in the hands of inexperienced workers, it is quite satisfactory for ordinary clinical purposes, if certain precautions are observed. It appears preferable

to determine the amount in the twenty-four hour stool, rather than that contained in a casual sample of bile, this also obviates the passage of the duodenal tube, a procedure which is often disagreeable to the patient

One case is cited to illustrate the value of urobilin estimations as an aid in the differential diagnosis of primary anemia from secondary anemia

An Italian, aged 51, entered the hospital in November, 1919, complaining chiefly of gastric distress and constipation which had lasted for two years, but which was never accompanied by real pain, vomiting or diarrhea. During the two weeks preceding admission, he had had a sudden attack of weakness and dizziness, followed by the appearance of tarry stools and shortness of breath. He had lost twenty-five pounds weight.

Physical Examination—Evidences of neuroretinitis were noted in both eyes, occurring in an anemic man, measuring about 5½ feet and weighing 143 pounds. Roentgenologic examination of the alimentary canal and sigmoidoscopy was negative.

Laboratory Findings—2,000,000 red blood cells, 40 per cent hemoglobin, 6,800 white blood cells, of which 58 per cent were polymorphonuclear. The blood smear showed irregularity in size and shape of the red cells, with central pallor and polychromatophilia on one occasion. The Wassermann test was negative. The gastric test meal contained no free hydrochloric acid, a total acidity of 32, no lactic acid and occult blood. The stool was repeatedly examined, it contained occult blood on one occasion only. It was never tarry. *The urobilin of the stool was persistently subnormal, there was none in the urine.*

The patient received two blood transfusions and was discharged after one month's stay in the hospital, with the diagnosis of pernicious anemia. This diagnosis was made largely because of the negative roentgen-ray findings.

Course—During the following six months, the patient gradually gained fifteen pounds in weight. His blood now contained the normal number of red blood cells and 70 per cent hemoglobin.

He was readmitted in June, 1920, because of the uncertainty of the diagnosis and because his gastric symptoms had increased in severity. The red blood cells now numbered 5,200,000, and the hemoglobin was 80 per cent. *The twenty-four hour quantity of stool contained only 1,760 dilutions of urobilin, the urine contained 400 dilutions on one occasion and 1,088 on another.*

Fluoroscopy now showed a mass in the region of the cardiac end of the stomach and this was confirmed by an exploratory laparotomy which further revealed metastatic involvement of the liver and retroperitoneal lymph nodes.

In this case, the severe anemia during the earlier period of the disease was probably due to a profuse hemorrhage from the tumor. The low urobilin content of the stool was against pernicious anemia and in favor of a new growth. The late occurrence of urobilinuria was the result of involvement of the liver.

During the past two years, urobilin estimations of the urine and stool have been carried out on about 100 patients. The results are tabulated under three headings.

Table 1 is a record of figures obtained in a group of patients not suffering from pernicious anemia or straightforward liver disease. With few exceptions, the figures are within normal limits. Patient

44,545, though regarded as a case leukemia, was suffering from acute anemia at one time and during this period the urobilin in the stool was much increased. Patient 45,815 was suffering from sprue, a disease having several features in common with pernicious anemia, such as an anemia with a high hemoglobin index, sore tongue and achylia and the high urobilin excretion suggests an increased rate of blood destruction. Bahr¹³ has encountered urobilinuria in several cases of sprue.

TABLE 1—RESULTS OF EXAMINATION OF CASES OF MISCELLANEOUS DISEASES

History Number	Diagnosis	Red Blood Cells, Millions per Cmm	Hemo-globin, per Cent	Urobilin Dilution Units	
				Urine	Feces
43957	Viridans endocarditis	2.9	60	Trace	7200
44051	Malaria	3.6	50	Negative	8000
44057	Leukemia	1.6	48	Negative	1400
44203	Carcinoma pancreas	4.1	70	Negative	Trace (jaundice)
44193	Bronchopneumonia	3.9	70	Negative	4400
44322	Nephritis	2.9	75	Negative	8000
44545	Leukemia	2.2	29	2160	Trace 18000 (blood crisis)
44387	Chronic bronchitis	5.6	70	Negative	6000, 7600
45175	Undiagnosed	4.7	78	Negative	6000
45233	Chronic nephritis	1.5	35	Negative	10000
45404	Carcinoma stomach	2.3	21	Trace	800
45297	Chronic cardiac disease	4.3	80	Negative	4800
46073	Dilatation of duodenum	5.2	85	Negative	6400
31655	Chronic colitis dilatation of colon, pilebitis	4.5	85	Negative, 330	Trace, trace
45772	Perinephric abscess	4.3	74	Negative	4800
45815	Psoriasis, hypertension	2.9	68	Negative	16000
44031	Carcinoma stomach, liver	2.5	35	Negative, 412, 1088	3200, 1760
46250	Purpura	1.8	22	590	20000
46612	Leukemia acute myeloid	1.3	20	863	
46642	Chronic nephritis, arteriosclerosis	3.1	50	Negative	3200
47168	Splenic anemia	3.4	50	976, 853, 732	25000, 19000
46678	Splenic anemia	2.4	65	612, 288, 320	4000, 1632, trace
47366	Carcinoma stomach	2.5	30	300	4000
46538	Syphilis	3.5	35		8000
46170	Syphilis	5.2	80	Negative	(jaundice) 3200
47815	Hypertension, syphilis	6.0	85	Negative	8000
48157	Chronic cardiac disease	5.1	90	Negative	3600, 12800
48753	Polycythemia	8.0	100	4160	
48321	Viridans endocarditis	3.3	65	Negative	6400
48420	Hodgkin's disease	4.2	75	Negative	2400
48092	Chronic nephritis		80	444	38400, 4800
48104	Hematuria	3.6	48	Negative	9600
48271	Ulcer of duodenum	1.8	28	Negative	8000
46838	Paroxysm, hemoglobinuria	4.4	70	Negative, trace	12800, 3200, 2720
31413	Purpura, streptococcus throat	5.6	75	432	4800
48631	Carcinoma peritonitis	4.3	44	340	3200
49381	Carcinoma peritonitis	4.8	55	Trace	6400

The increased urobilin excretion observed in patient 46,250, suffering from purpura, was probably due to the rapid disintegration of the extravasated red cells. In the case of splenic anemia (47,108), it is difficult to determine whether the high excretion was due to increased hemolysis or to involvement of the liver.

13 Bahr, P. H. A Report on Researches in Sprue in Ceylon, 1912-1914

TABLE 2—UROBILIN EXCRETION IN HEPATIC DISEASES

History Number	Diagnosis	Red Blood Cells, Millions per Cmm	Hemoglobin, per Cent	Urobilin Dilution Units	
				Urine	Feces
42640	Cirrhosis	4 2		188	2400
41796	Cirrhosis, cholelithiasis	4 5	92		1000 (jaundice)
45179	Cirrhosis	4 0	80	2400, 1360, 1200	22000, 16000
45247	Cirrhosis	5 5	80	1620	8000
45511	Cirrhosis	4 5	95	2536	3200
46114	Cirrhosis	4 0	70	1890	11400
47493	Cirrhosis	6 0	83	1740	16000
46895	Cirrhosis	4 1		Neg, Neg	4600, 6400
46927	Cirrhosis		80	2000	16000
45434	Abscess	5 0	70	1740	
45367	Carcinoma	4 7	80	5200, 4760	4000
45855	Carcinoma	4 6	80	500, 376, trace	40000, 24000
47108	Carcinoma			1568, 1400	19800, 8000
45415	Carcinoma stomach, cirrhosis ?	5 1	65	564	3840
33081	Cholelithiasis	4 8	80		16000
46983	Cholelithiasis	4 8	90	1320, 396	2400 (jaundice)
45493	Congestion	3 3	58	4120	
47283	Congestion	5 2	70	2576	2460
47135	Catarrhal jaundice during convalescence				16000
44496	Catarrhal jaundice	3 2	70	Negative	2400
47171	Syphilis liver, spleen	4 6	42	1236, 2432	33000 21120, 25000
45103	Carcinoma			320	16000
49499	Hodgkin's disease	4 2		3136	8000
32084	Cirrhosis	5 5	85	Negative	8000 12500
48252	Syphilis liver	5 0	80	Negative	18000
48155	Carcinoma	5 8	90	672, 624, 756	16000
49045	Syphilis	4 3	80	1200	12500
48644	Cirrhosis	3 7	70	1600	21200
48921	"Salvarsan" jaundice	4 0	65	552	6400
48165	Catarrhal jaundice	5 0	110	1944	
48733	"Salvarsan" jaundice	5 0	75	Neg, Neg	4500, trace
46170	"Salvarsan" jaundice			992, Neg, 600	800, 20000, 12800
30510	Jaundice, cholelithiasis	4 8	90	960	6400
48052	Carcinoma	4 2	90	1568	12500
49330	Jaundice (cause ?)	3 5	70	Negative	1600
47998	Tumor	6 8	80		6400
49414	Splenomegaly, anemia	4 5	59	Trace	4000, 3200
49195	Congestion	4 4	70	1280	8000
48797	Syphilis			Negative	

TABLE 3—UROBILIN IN CASES OF PERNICIOUS ANEMIA

History Number	Red Blood Cells Millions per Cmm	Hemoglobin, per Cent	Urobilin Dilution Units	
			Urine	Feces
44321	2 2	70		72000
44433	1 3	35		32000
44572	1 7, 1 5	35, 50	544	
44657			Trace, 1056	12000, 40000
40643	3 8	60	Negative, 1524	20000, 36000
44559	2 0	50	Negative	20000
45190	2 0	45	2200, negative	32000, 16000
45398	1 1	35	1120, 500	20000, 22000
45506	1 4	50	400, trace	18000, 12000
45596	3 4	80	2640	24000
45967	3 0	38	1520	21200
37456	1 6	40	668 990	17760 12800
46587	1 9	45	1044, 1118, 680	32000, 25600 24000
45773	1 0, 1 2	20, 25	3000	20000, 16000
47589	2 0	45	1740, 7952, 1588	20000, 24000, 16000, 40000
47317	1 6	46	600	32000, 40000
48124	0 8	25	864	16000, 22000
45398	1 7	20	640, 200, 960	25000, 33260
45559	0 8, 1 6	30, 40	966, trace	100000 40000
46164	1 9	45	Trace, negative	26400, 16000, 32000
46498	2 0	40	1944	40000
48640	1 2	38	672	40000
48360	1 7	70	1600	21200
47780	0 8	30	588, 800	16000, 24000
			1650, 1620	31800, 12000

Patient 48,092 was suffering from chronic nephritis of the retention type and secondary anemia. The high urobilin excretion was apparently due to the hemolytic activity of the retained waste products.

Patient 46,838, regarded as having paroxysmal hemoglobinuria, did not have an attack during the period of observation.

In the diseases of the liver, we have usually found an increase of urobilin in the urine alone or in the stool alone, or in both (Table 2).

In pernicious anemia, a high urobilin value was obtained in every instance if care was taken in the collection of the stool, and if the estimation was repeated sufficiently often (Table 3).

A STUDY OF THE S-T INTERVAL IN ONE THOUSAND AND TWENTY-EIGHT ELECTROCARDIOGRAMS

J ARTHUR BUCHANAN, M D

ROCHESTER, MINN

The interval of the electrocardiogram from the termination of the S wave to the end of the T wave is being investigated by various observers in order to establish the method of its production and the significance of the variations from the normal. This interval is designated the S-T interval.

The present interpretation of the phenomena of the S-T interval rests, fundamentally, on the observations of a few workers. The foremost among these is Galvani,¹ who, in 1786, discovered electric currents in the muscles of the frog. The flow of electric current from points of higher potential to points of lower potential was first observed by Nobili,² in 1824, shortly after his invention of the galvanometer. Kolliker and Mueller,³ in 1855, while pursuing investigations similar to those of Galvani, found each beat of the frog's heart to be accompanied by a definite electric current. The next great advance was made by Waller,⁴ in 1887, who succeeded in demonstrating the electric changes that accompany contraction of the cardiac muscle in intact animals and in man by the use of the capillary electrometer. During these investigations Waller was able to record the distribution in the remainder of the body of the electropotential which accompanies the beats of the human heart.

The final steps in the evolution of a means of graphically representing changes in electropotential during cardiac activity were made by Ader,⁵ in 1897, and Einthoven,⁶ in 1903. The invention of the string

1 Galvani, L. *The Encyclopedia Britannica* Ed 11, Cambridge, Cambridge Univ Press **11** 428, 1910

2 Nobili, L. *The Encyclopedia Britannica* Ed 11, Cambridge, Cambridge Univ Press **19** 724, 1911

3 von Kolliker, R A, and Mueller, H. *Nachweis der negativen Schwankung am natürlich sich kontrahierenden Muskel* Verhandl d physik-med Gesellsch, Würzburg **6** 528, 1855

4 Waller, A D. *A Demonstration on Man of Electromotive Changes Accompanying the Heart's Beat*, *J Physiol* **8** 229, 1887

5 Ader, C. *Sur un nouvel appareil enregistreur pour cables sous-marines*, *Compt rend Acad d sc* **124** 1440, 1897, Crile, G W. *An Electro-Chemical Theory of Normal and Certain Pathologic Processes with Clinical Application* Mayo Foundation Lecture, January 11, 1921. Unpublished

6 Einthoven, W. *Ein neues Galvanometer*, *Ann d Physik* **12** 1059, 1903. *Ueber einige Anwendungen der Seitengalvanometer* *Ann d Physik* **14** 182, 1904

galvanometer by the former made the quantitative measurement of electropotential possible. The photographic apparatus added to Ader's instrument by Einthoven resulted in the electrocardiograph essentially as it exists today.

In attempts to reach a convincing explanation of the phenomena in the electrocardiogram which are graphically expressed in the interval extending from the termination of the S wave to the end of the T wave, various ideas have been presented. In a recent article Willius⁷ reviewed the noteworthy contributions on this subject. His studies and investigations led him to believe that the horizontal portion, not infrequently absent, of the S-T interval represents an iso-electric state of the heart. The T wave, or the final portion of this interval, he believes to be the expression of preponderance of contraction on one side of the line of equipotential. Lewis,⁸ however, does not believe that the problem has been definitely settled.

Variation in the length of the S-T interval has received slight consideration. Meakins⁹ discussed his findings in a small number of cases. My own study consists of an analysis of 1,028 electrocardiograms, from 924 patients, made in the Mayo Clinic from Jan. 1, 1918, to June 1, 1918, and was undertaken in order to determine the frequency and associations of variations in the length of the S-T interval.

For clarity and brevity, the term "sinus rhythm" is used in the Mayo Clinic to designate all regular hearts with rates between 70 and 90 beats each minute, "sinus bradycardia" signifies all regular hearts with a rate below 70 beats each minute, while the term "sinus tachycardia" is used in all cases in which, with a regular heart, the rate is above 90 beats each minute, where the impulse originates in the normal pacemaker.

Normal S-T Interval—The normal interval has been estimated at 0.28 second. Sixty-nine of the electrocardiograms (6.72 per cent) recording sinus rhythm in normal hearts showed an average length of 0.28 second in all three derivations. Nine (13.04 per cent) recorded prolonged S-T intervals, ranging from 0.29 to 0.34 second in any derivation, with an average for all derivations of 0.31 second. The shortest time interval for the S-T interval to be considered normal was 0.24 second. Two (2.89 per cent) of the electrocardiograms recorded a shortened S-T interval. The average interval for all derivations in these two electrocardiograms was 0.20 second. Clinically, there was nothing to substantiate a diagnosis indicative of a cardiac

7 Willius, F. A. Observations on Negativity of the Final Ventricular T Wave of the Electrocardiogram, *Am J M Sc* **160** 844, 1920.

8 Lewis, T. The Mechanism and Graphic Registration of the Heart Beat. London, Shaw and Son, 1920, pp. 108-116.

9 Meakins, J. Prolongation of the "S-T" Interval of the Ventricular Complex as Shown by the Electrocardiograph. *Arch Int Med* **24** 489 (Oct.) 1919.

lesion in the patients showing a prolonged or a shortened S-T interval, yet it is, perhaps, correct to suspect, because of its rarity in the normal heart and its frequency in the diseased heart, that it really speaks for some underlying, although yet undetermined, disturbance

S-T Interval with Sinus Tachycardia—In this group, 148 (14.39 per cent) of the electrocardiograms were made from patients with normal rapid hearts. The average interval for all three derivations was 0.24 second. Six (4.05 per cent) showed a prolongation of the interval ranging from 0.30 to 0.32 second in any derivation, or an average interval for all derivations of 0.31 second. The interval was found to be shortened in twenty-nine (19.59 per cent). The interval ranged from 0.16 to 0.22 second in any derivation, with an average length for all derivations of 0.19 second.

Abnormal Hearts with Sinus Rhythm—In this group were recorded 143 (13.91 per cent) electrocardiograms from patients with intrinsic and extrinsic cardiac disease with normal cardiac rate. The average time for the S-T interval in all derivations was 0.30 second, or a slightly increased time interval for the whole series. Thirty-one (21.67 per cent) showed a prolonged time interval. The average longest interval for any derivation was 0.36 second, and the average shortest was 0.30 second. In the patients with myocardial changes who showed prolonged intervals were associated (1) myocardial degeneration with hypertension, nine patients, one with mild decompensation, (2) aortic regurgitation, four patients, (3) combined aortic and mitral valve regurgitation, one patient, (4) mitral regurgitation, four patients; (5) mitral stenosis, one patient, (6) combined mitral lesions, two patients, (7) general arteriosclerosis, six patients, (8) coronary sclerosis, three patients, and (9) dextrocardia, one patient. The rarity of shortened S-T interval in the moderately slow heart is evidenced by three (2.09 per cent) electrocardiograms, which showed an average length of time for the S-T interval in all derivations of 0.21 second. The myocardial changes showing a shortened interval were associated in one case with aortic regurgitation, in one with arteriosclerosis, and in one with mitral regurgitation.

S-T Interval in Abnormal Heart with Sinus Tachycardia—Under this heading 132 (12.84 per cent) electrocardiograms were studied, and they showed an average time for the S-T interval in each derivation of 0.22 second. Rapid abnormal hearts as a group show a shortened S-T interval. The rarity of prolonged interval in the rapid abnormal heart was evidenced in four (3.03 per cent) electrocardiograms in which the average longest interval for any derivation was 0.31 second. The clinical diagnosis in three of these cases was myocardial disease with arteriosclerosis, and in the fourth the diagnosis was aortic regurgitation. A shortened interval was found in twenty-nine (21.96 per

cent) electrocardiograms, the longest interval for any derivation was 0 22 second, and the shortest was 0 16 second, with an average interval for all derivations of 0 18 second. The myocardial changes in the patients showing a shortened S-T interval in the electrocardiogram were associated with (1) exophthalmic goiter, ten patients, (2) arteriosclerosis, ten patients, (3) mitral stenosis, one patient, (4) combined mitral lesions, two patients, (5) mitral regurgitation, two patients, and (6) congenital heart disease, four patients.

Premature Ventricular Contractions — This phenomenon was observed in 143 (13 91 per cent) electrocardiograms, in which the average S-T interval for each derivation was 0 28 second. Prolongation of the S-T interval in electrocardiograms showing premature ventricular contractions occurred in twelve (8 39 per cent). The longest interval for any derivation was 0 40 second, and the shortest 0 30 second, with an average of 0 33 second for all derivations. A shortened interval with premature ventricular contractions was seen in three instances (1 39 per cent). The average interval for all derivations was 0 20 second. The myocardial changes in the patients showing premature ventricular contractions in the electrocardiograms were associated with (1) arteriosclerosis, fifty-seven patients, (2) myocardial degeneration and hypertension, three patients, (3) exophthalmic goiter, four patients, (4) auricular fibrillation, fourteen patients, (5) adenoma with hyperthyroidism, fifteen patients, (6) mitral stenosis, one patient, (7) mitral regurgitation, three patients, and (8) combined mitral lesion, one patient. In an undetermined group of forty-six patients with premature ventricular contractions no cardiovascular disease was found.

Premature Auricular Contractions — Fifty-five (5 35 per cent) electrocardiograms were studied in this group and showed an average length of time for the S-T interval in each derivation of 0 27 second. The S-T interval was prolonged in eleven (20 per cent) cases in this group, the longest interval for any derivation was 0 36 second, and the shortest interval for any derivation was 0 30 second, giving an average time for all derivations of 0 33 second. The association of premature auricular contraction with shortened S-T interval was observed in one electrocardiogram. The time interval was 0 20 second for all derivations. The myocardial changes in the cases presenting premature auricular contractions were associated with (1) adenoma with hyperthyroidism, five patients, (2) myocardial degeneration and hypertension, four patients, (3) arteriosclerosis, five patients, (4) mitral stenosis, one patient, (5) mitral regurgitation, two patients, (6) exophthalmic goiter, one patient, and (7) chorea, one patient. Premature auricular contractions were also found in an undetermined group of thirty-six cases.

Sinus Bradycardia in the Normal Heart—A ventricular rate of less than 70 beats each minute was found in forty-eight (4.66 per cent) electrocardiograms. The average time for the S-T interval for each derivation was 0.33 second. Slow hearts, as a group, show a prolonged S-T interval. In this series twenty-three (47.91 per cent) electrocardiograms showed a prolonged interval, the greatest length of time for any derivation was 0.36 second, and the shortest 0.30 second, giving an average interval for all derivations of 0.32 second. In no instance was a shortened S-T interval found in the normal, unusually slow heart.

Sinus Bradycardia in the Abnormal Heart—A ventricular rate of less than 70 beats each minute was found in fifty-five (5.35 per cent) of the electrocardiograms from abnormal hearts. The average S-T interval for each derivation was 0.31 second. Forty (72.72 per cent) electrocardiograms showed a prolonged S-T interval. The longest S-T interval in any derivation was 0.44 second, and the shortest 0.30 second, giving an average interval in all derivations of 0.36 second. A shortened interval did not occur in the slow, abnormal heart. The myocardial changes in the cases of sinus bradycardia showing a prolonged S-T interval were associated with (1) myocardial degeneration and hypertension, five patients, (2) arteriosclerosis, nineteen patients, (3) coronary sclerosis, five patients, (4) mitral regurgitation, four patients, (5) mitral stenosis, one patient, (6) combined mitral lesion, two patients, and (7) aortitis, two patients. A patient with septic tonsils without heart involvement presented a prolonged S-T interval with an unusually slow heart. No cases of decompensation were recorded.

Sinus Arrhythmia in the Normal Heart—This condition was found in twenty (1.9 per cent) electrocardiograms. The average S-T interval for each derivation was 0.29 second. A prolonged S-T interval was observed in nine (45 per cent) electrocardiograms. The longest interval for any derivation was 0.36 second, and the shortest 0.32 second. The average interval for all derivations was 0.34 second. A shortened interval was not observed.

Sinus Arrhythmia in the Abnormal Heart—Fifteen (1.45 per cent) electrocardiograms in this group showed an average S-T interval for each derivation of 0.28 second. A prolonged interval was found in eight (53.33 per cent) electrocardiograms. The longest interval for any derivation was 0.36 second, and the shortest 0.32 second, giving an average interval for all derivations of 0.34 second. A shortened interval was found in two (13.33 per cent) electrocardiograms. The S-T interval for all derivations was 0.22 second. The myocardial changes in the cases presenting a prolonged S-T interval were associated with (1) aortic regurgitation, one patient, (2) coronary sclerosis, one

patient, (3) myocardial degeneration and hypertension, three patients, (4) arteriosclerosis, one patient, (5) congenital heart disease, one patient, and (6) mitral regurgitation, one patient. The myocardial changes in the group presenting a shortened S-T interval were associated with general arteriosclerosis, one patient, and attacks of paroxysmal tachycardia, one patient.

Auricular Fibrillation—Eighty-one (78.8 per cent) electrocardiograms were studied under this heading. The average S-T interval for all derivations was 0.22 second. A prolonged interval was observed in three instances (3.70 per cent). The average time interval for all derivations was 0.33 second. A shortened interval was observed in thirty-three (40.74 per cent) electrocardiograms. The longest

TABLE 1—COMPARATIVE FREQUENCY OF PROLONGED S-T INTERVAL

Cases	Normal hearts	Percentage prolonged
23	Sinus bradycardia 47	
9	Sinus rhythm 13	
9	Sinus arrhythmia 4	
6	Sinus tachycardia 4	
Abnormal hearts		
40	Sinus bradycardia 72	
8	Sinus arrhythmia 53	
2	Heart block 40	
97	Left ventricular preponderance 22	
31	Sinus rhythm 21	
11	Auricular extrasystoles 20	
22	Right ventricular preponderance 13	
12	Ventricular extrasystoles 8	
4	Aberrant Q R S Complexes 6	
4	Sinus tachycardia 3	
3	Auricular fibrillation	

interval for any derivation in this group was 0.22 second, and the shortest 0.12 second, giving an average for all derivations of 0.20 second. A group study of auricular fibrillation shows a shortened S-T interval.

Left Ventricular Preponderance—This group, as well as that of right ventricular preponderance, is included in Tables 1 and 2 with the abnormal hearts, not because all the cases with preponderance were found associated with recognizable abnormal hearts, but for purposes of group study. Left ventricular preponderance was observed in 434 (42.21 per cent) electrocardiograms. The average S-T interval for each derivation was 0.26 second. Ninety-seven (22.32 per cent) showed a prolonged interval, the longest time period for any derivation was 0.40 second, and the shortest 0.30 second, making an average

for all derivations of 0.30 second. A shortened interval was found in thirty-five (80.6 per cent) electrocardiograms. The longest period for any derivation was 0.22 second, and the shortest 0.16 second, making an average for all derivations of 0.21 second.

Right Ventricular Preponderance—One hundred sixty-six (16.05 per cent) electrocardiograms were studied in this group. The average time interval for all derivations was 0.25 second. Twenty-two (13.33 per cent) electrocardiograms showed a prolonged interval, the longest time for any derivation was 0.36 second, and the shortest 0.30 second, making an average for all derivations of 0.32 second. A shortened interval was found in thirty-three (20 per cent) electrocardiograms. The longest interval for any derivation was 0.22 second, and the shortest 0.21 second.

TABLE 2—COMPARATIVE FREQUENCY OF SHORTENED S-T INTERVAL

Cases	Normal hearts	Percentage prolonged
29	Sinus tachycardia	19
2	Sinus rhythm	2
0	Sinus bradycardia	0
0	Sinus arrhythmia	0
Abnormal hearts		
33	Auricular fibrillation	40
29	Sinus tachycardia	21
1	Partial heart block	20
12	Aberrant Q R S complexes	20
33	Right ventricular preponderance	19
2	Sinus arrhythmia	13
35	Left ventricular preponderance	8
3	Ventricular extrasystoles	2
3	Sinus rhythm	2
1	Auricular extrasystoles	0.9
0	Sinus bradycardia	0

Angina Pectoris—Electrocardiograms were made from twenty-seven (26.2 per cent) patients with angina pectoris. The cases with myocardial changes presented the following conditions: (1) general arteriosclerosis, seven patients; (2) cardiac hypertrophy with mitral regurgitation, three patients; (3) syphilitic aortitis, one patient; and (4) coronary sclerosis, nine patients. In a group of seven patients no cardiac lesion could be determined. The average time for the S-T interval for all the patients in all derivations was 0.26 second. Only three patients (11.11 per cent) showed prolongation of the S-T interval with an average for all derivations of 0.32 second. Two (7.44 per cent) patients showed a shortened S-T interval with an average for all derivations of 0.18 second. The systolic blood pressure

was increased in eighteen (66.66 per cent), and the diastolic was above normal in twenty-one (77.77 per cent). The findings in this group make it possible to state that there is no connection between the pain of this affection and variations in the length of the S-T interval. All the patients were subjected to a period of exercise after which a second electrocardiogram was made, this did not differ from the primary record.

Aberrant Q R S Complexes in all Derivations—Sixty (58.3 per cent) electrocardiograms in this group showed an average S-T interval for each derivation of 0.20 second. A prolonged interval was found in four (6.66 per cent) electrocardiograms. The longest interval for any derivation was 0.40 second, and the shortest 0.32 second, making an average for all derivations of 0.36 second. In the twelve (20 per cent) electrocardiograms with shortened interval the longest interval for any derivation was 0.20 second, and the shortest 0.16 second, making an average for all derivations of 0.19 second.

TABLE 3—AGE OF PATIENTS, IN DECADES, MANIFESTING CHANGE IN S-T INTERVAL

Decades	Patients With Prolonged S-T Interval	Patients With Shortened S-T Interval
0 to 10	0	9
11 to 20	10	9
21 to 30	27	37
31 to 40	64	37
41 to 50	63	43
51 to 60	64	31
61 to 70	50	17
71 to 80	9	2

Partial or Complete Heart Block—There were five patients in this group which showed an average S-T interval for each derivation of 0.27 second. Of these patients two (40 per cent) showed an average interval for all derivations of 0.32 second, while one electrocardiogram showed an interval of 0.21 second in Derivation I, 0.24 second in Derivation II, and 0.20 second in Derivation III.

Sex and Age—The 287 (27.91 per cent) electrocardiograms which showed a prolonged S-T interval were made from 175 (60.97 per cent) males, and 112 (39.03 per cent) females. One hundred eighty-five (17.99 per cent) electrocardiograms which showed shortening of the S-T interval were made from seventy-four (40 per cent) males, and 111 (60 per cent) females. The higher percentage of females in the group showing shortened S-T interval is due to the greater frequency of exophthalmic goiter in females.

Blood Pressure—A study was made of each group of the systolic and diastolic blood pressures in order to determine if there was any relation between variations in blood pressure and the length of the

S-T interval The unimportance of blood pressure in variations in the length of the S-T interval is shown in Table 4

T Wave Negativity—This phenomenon was observed in 511 (49.70 per cent) electrocardiograms in various combinations and in various derivations. It is interesting to note that, as in the studies of Willius, T wave negativity was never found in Derivation II alone or in combined Derivations I and III. The S-T interval was found prolonged in Derivation I with T wave negativity in six patients, and in forty-two (8 per cent) patients in Derivation III. In combination the S-T interval was found prolonged in association with T wave negativity once in Derivations I and II, twice in Derivations I, II and III, and three times in Derivations II and III. The S-T interval was shortened in Derivation I three times in association with T wave

TABLE 4—RELATION OF BLOOD PRESSURE TO S-T INTERVAL

	Normal Hearts		Abnormal Hearts		Normal Hearts				Abnormal Hearts			
					Prolonged S-T Interval		Shortened S-T Interval		Prolonged S-T Interval		Shortened S-T Interval	
	Sys tolie	Dias tolie	Sys tolie	Dias tolie	Sys tolie	Dias tolie	Sys tolie	Dias tolie	Sys tolie	Dias tolie	Sys tolie	Dias tolie
Sinus rhythm	128	79	148	77	133	80	130	76	140	80	163	49
Sinus tachycardia	140	73	132	68	151	85	153	86	134	85	141	65
Sinus bradycardia	123	72	135	78	127	77			146	77		
Sinus arrhythmia	113	73	128	73	117	70			135	72	125	81
Auricular fibrillation			114	63					133	63	130	79
Left ventricular preponderance			145	81					144	84	140	75
Right ventricular preponderance			126	74					132	78	197	116
Angina pectoris			151	83					148	103	160	80
Aberrant Q R S complex			152	86					147	86	162	75

negativity, and twenty-two (4 per cent) times in Derivation III. The interval was found shortened with T wave negativity in combined Derivations I and II, six times, in Derivations I, II, and III, six times, and in Derivations II and III, six times.

Exophthalmic Goiter—Electrocardiograms were made from eighty-one (78.7 per cent) patients. Forty-two (51.85 per cent) electrocardiograms showed a normal S-T interval, three (3.71 per cent) a prolonged interval, thirty-three (40.61 per cent) a shortened period, and in three (3.71 per cent) the interval could not be read because of auricular fibrillation in which the irregularity was such as to obscure the definition of the T wave. The frequency of shortened S-T interval in exophthalmic goiter is sharply contrasted with the infrequency of this finding in cases of adenoma with hyperthyroidism. In the latter condition, the S-T interval was shortened six times (16.21 per cent) in patients with auricular fibrillation, once in a patient with simple

sinus tachycardia, it was prolonged in six (16.21 per cent) patients. Thirty-seven (35.9 per cent) electrocardiograms were made from patients with adenomas with hyperthyroidism.

DISCUSSION

Variations in the length of the S-T interval are common. Of the 1,028 electrocardiograms studied a total of 472 (44 per cent) showed an abnormal length of the interval. Prolongation (27 per cent) is more frequently observed than shortening (18 per cent). The comparative frequency of changes in the length of the interval is shown in Tables 1 and 2. The data presented demonstrate the lack of a constant relationship of variation in the length of the interval with any other phenomena in the electrocardiogram. It is to be noted, however, that prolongation is found more often in slow hearts, while shortening is found more often in the rapid heart. There is an intimate relationship between cardiac rate and length of the S-T interval.

The pathologic conditions associated with variations in the length of the S-T interval are diverse. A study of the clinical diagnosis clearly shows that there is no organic lesion of the cardiac structures which is constantly associated with a variation in the length of the S-T interval. A prolonged interval is found in electrocardiograms made from hearts, which, although diseased from an anatomic standpoint, are usually capable of functioning properly. The reverse holds for the shortened interval which is associated with cardiac lesions which have eventuated into more or less marked cardiac distress. Shortening, nevertheless, is not always found in hearts that are functionally embarrassed.

It might be suspected that interference with the conduction apparatus would have a constant bearing on the length of the S-T interval. This does not hold true. There are no constant changes in cases of partial or complete block. Lesions affecting the interventricular ramifications of the conduction bundle, which present themselves as aberrant Q R S complexes, are also not associated with distinctive variations in the length of the S-T interval. This deduction is based on the forty-four (74 per cent) electrocardiograms of this type which showed no variation in the length of the S-T interval. Partial or complete heart block and aberrant Q R S complexes are the expression of involvement of the conduction apparatus before branching and very shortly after branching, so that it can be definitely stated that lesions of the conduction system have no distinctive influence in variations in the length of the S-T interval.

It is safe to believe that no anatomic condition of the heart can cause, per se, variation in the length of the S-T interval. This belief, which is borne out by the data of this study, must be correct if the

theory of electric potential is correct. If the horizontal portion of the S-T interval is the expression of an iso-electric state, and if the T wave is the expression of preponderance of contraction on one side of the line of equipotential, it follows that only some factor which either accelerates or retards the rate of change in electropotential can cause a variation in the length of the S-T interval. This factor is not brought out by my investigations. It is, no doubt, electrochemic in nature rather than histopathologic. This assumption is suggested by the electrochemic theory of living matter recently advanced by Crile.

CONCLUSIONS

- 1 The normal time period of the S-T interval ranges from 0.24 second to 0.28 second
- 2 Variations in the length of the S-T interval are common
- 3 Variations are independent of anatomic lesions
- 4 The length of the S-T interval is more dependent on cardiac rate than any other factor
- 5 Variations in the length of the S-T interval are not a constant accompaniment of any of the other phenomena of the electrocardiograms
- 6 Males present variations in the length of the S-T interval slightly more often than females
- 7 Variations in the length of the S-T interval are suggestive of retardation or acceleration of the rate of change in electropotential

PERFORATION OF THE INTERVENTRICULAR SEPTUM OF THE HEART

WITH REPORT OF A CASE*

JOHN B YOUMANS, M D
BOSTON

Perforation of the interventricular septum of the heart, aside from cases of congenital malformation, in the great majority of cases is due either to rupture of an interventricular aneurysm or to ulcerative endocarditis. Perforation from other causes, such as rupture of a hydatid cyst,¹ has been described but such cases are relatively very rare.

CARDIAC ANEURYSMS

Cardiac aneurysms have long been the subject of reports in the literature, and many excellent monographs have been written on the subject. It is said to have been first described by Guzman Galeati in 1757.² Also Thurman³ reports a case from the manuscripts of John Hunter described in 1757. The first monograph was written by Breschet in 1827.⁴ Pelvet,⁵ in 1867, gave an excellent discussion of the subject. Bourland,⁶ in 1904, without giving references, reports having collected 147 cases from the literature. Osler and McCrae⁷ refer to 135 cases, and Friedlander and Isaacs⁸ reported a case recently.

Aneurysms of the heart may form in the valves, walls or interventricular septum. They may be acute or chronic. Acute aneurysms are associated with acute endocarditis and will be considered under the discussion of that condition. Chronic aneurysms are, in the great majority of cases, the result of chronic myocarditis and fibrosis. As a rule, the chronic myocarditis is due to arteriosclerosis of the coronaries, and the anterior branch is the one most commonly affected. The left ventricular wall, near the apex, is the most usual site, as would logically follow as the result of the anterior coronary being most often affected. The aneurysmal formation may vary from the slightest aneurysmal

*From the Medical Service Massachusetts General Hospital

1 Crowther, B. *Austral M J* **2** 362, 1880

2 Cited by Pelvet

3 Cited by Pelvet

4 Cited by Pelvet

5 Pelvet. *Des Aneurysmes du Coeur*, Paris Delchage, 1867

6 Bourland. *Aneurysm of the Heart*, *Am J M Sc* **128** 323, 1904

7 Osler and McCrae. *Modern Medicine*, Ed 2, Philadelphia, W B Saunders Company, **4** 482, 1915

8 Friedlander, A, and Isaacs, R. *Interventricular Cardiac Aneurysm with Heart Block*, *J A M A* **75** 1778 (Dec 25) 1920

dilatation to a definite aneurysmal sac. The wall is thinned and the muscle tissue is largely replaced by fibrous tissue. There frequently are adhesions to the pericardium and rupture is fairly common.

Aneurysms of the interventricular septum differ in no essential respect from cardiac aneurysms in general, except as to location. According to Klein⁹ they were not described until 1839. In contradistinction to cardiac aneurysms in general, they are most commonly found at the base of the heart, in the "undefended space" or *pars membranacea*. As the whole septum derives its blood supply from the anterior coronary artery which is most commonly involved in cardiac aneurysms, some additional reason has been sought for the occurrence of septal aneurysms at the base in the large majority of cases. Klein mentions gross abnormalities and increased pressure in the left ventricle, due to hypertrophy, acting on the weakest part of the wall. Others have discussed the character of the tissue forming this part of the septum which is fibrous in character. Also endocarditis is more frequent in this region due to easy extension from the valves, causing the formation of a weak spot and a subsequent aneurysm.

PERFORATION OF THE INTERVENTRICULAR SEPTUM

Perforation of the interventricular septum due to ulcerative endocarditis has also been reported fairly frequently. Callender¹⁰ reported a case in 1858, while Fournier,¹¹ in 1884, discussed the condition at length and reported a case of his own and seven others which he collected. Gennari,¹² in 1904, reported a case and gave a short bibliography.

For reasons which are similar to those given for the more frequent occurrence of septum aneurysms in the *pars membranacea*, perforation due to ulcerative endocarditis is also most likely to occur in that area. Proximity to the valves from which involvement often occurs by direct extension, the character of the tissue, the poor blood supply in that area, congenital defects, all make this area peculiarly susceptible to such an infection with resultant perforation.

The symptoms of perforation of the interventricular septum are indefinite and are not pathognomonic of the condition. In the cases of perforation of fair size, occurring acutely, there is the picture of sudden cardiac failure. When, as in the majority of cases, it occurs more

9 Klein, G. Zur Aetiologie der Aneurysmen der *pars membranacea septi Ventriculorum Cordis* and deren Ruptur, *Arch f path Anat* **118** 57, 1889.

10 Callender. *Med Times & Gaz* Feb 1858.

11 Fournier, H. C. *Etude sur les Perforations de la Cloison interventriculaire dans l'Endocardite Ulcereuse*, Paris, 1884, Davy.

12 Gennari, C. *Sopra un Caso di perforazione del setto interventriculaire determinata da endocardite ulcerosa*. *Riv Crit di clin med* **5** 717, 1904.

gradually, there is only a greater or smaller increase in the signs of cardiac distress which the patient already shows. The murmurs are not characteristic. Fournier speaks of cyanosis as an important sign but in Talamon's¹³ case there was no cyanosis. Gennari says that the most important signs are pulsation to the right of the sternum, dilatation of the right ventricle, a murmur to the right of the sternum transmitted to the right and a thrill in the same area.

While cardiac aneurysms are fairly common, aneurysms of the interventricular septum are rather rare and perforation of the septum due to their rupture is even more rare. Including cases of perforation of the septum due to ulcerative endocarditis, a careful search of the literature since 1879 has yielded but twenty-two cases of perforation of the septum due to all causes, which were reported as such. Peacock¹⁴ in 1854, reported a case in which the rupture also included the ventricular wall. Reinhardt,¹⁵ in 1857, reported a case due to rupture of an aneurysm of the interventricular septum, Talamon cited a case in 1878, V. Buhl¹⁶ has reported five cases and Klein and Lee¹⁷ each one. Callender apparently reported the first case of perforation due to ulcerative endocarditis in 1858. Fournier, in 1884, reported one case of his own and seven others. Peron,¹⁸ Fisher¹⁹ and Gennari have each reported one case. In all of these cases the perforation occurred at the base of the septum and in none of them was there found a perforation similar to the one occurring in the case to be described below, in which case the perforation occurred near the tip of the septum a short distance above the apex.

REPORT OF CASE

History—R. B. (East Medical 238,660), a widowed, white woman, aged 52, entered the Massachusetts General Hospital Sept. 13, 1920. She was born in Pennsylvania and had lived in Massachusetts forty-seven years. Her family, marital and social histories were essentially negative. According to her past history, her general health had been good until about ten years previously.

13 Talamon, C. Endocardite du Septum et Aneurysme interventriculaire, perforation de la Cloison, endocardite de l'infundibulum, *Progres méd* 7 984, 1878.

14 Peacock. *Tr Path Soc Lond* 5 102, 1854.

15 Reinhardt. *Zur Anatomie und Pathologie der dunnen Stelle in der Herzcheidewand*, *Arch f path anat* 12 129, 1857.

16 Buhl. *Ztschr f Biol* 253, 1880.

17 Lee, A. E. Case of ruptured aneurysm in the Ventricular Septum with Sudden Death, *U S Naval Bull* 2 49, 1908.

18 Peron, A. Endocardite droit infectieuse post-peurperil, perforation de la Cloison interventriculaire par une lesion unique ayant amene une insuffisance tricuspideenne et insuffisance aortique, *Bull Soc Anat de Par* 70 198, 1895.

19 Fisher, H. A case of Ulcerative Endocarditis involving the Pulmonary Valve, causing a perforation of the ventricular septum, gangrene of the nose and ears and multiple infarcts of the kidneys, *Proc Path Soc Phila* 6 98, 1902.

Since then she had had frequent tonsillitis. She had entered this hospital on three previous occasions. The first time (East Surgical 204,937), in the fall of 1915, she complained of several attacks of epigastric pain, nausea, vomiting, headache, clay colored stools, dysuria, and frequency of urination. Operation showed only a slightly inflamed appendix which was removed. Her second entry a year later (East Surgical 210,450), was for a similar complaint plus backache and a vaginal discharge. At this time the urine showed sugar on two of five examinations and albumin three times. At her third entry, two years later (East Surgical 282,282), she complained of the same right upper quadrant pain plus moderate dyspnea on exertion and nocturia. Physical examination showed the heart not enlarged and slight sinus arrhythmia. The urine showed 20, 0, and 29 per cent sugar, respectively, on three examinations. Following her discharge she was treated in the diabetic clinic of the outpatient department for about a year. She was placed on a restricted diet and did well, her urine on the last day she attended the clinic showing only the slightest possible trace of sugar.

On her present admission she stated that she had had slight dyspnea with mild anginoid pains, and slight palpitation when tired or excited, occasional orthopnea and edema for eight or ten years, and a dry, unproductive cough for years. She had been on a general diet since she had left the outpatient department eighteen months previously.

Present Illness—The present illness occurred suddenly, ten days before admission, with substernal distress during moderate exertion, which had passed off in about twenty minutes. That evening, while in bed, she had a sudden, sharp, substernal pain, radiating to the back, shoulders and left arm. It was associated with dyspnea and orthopnea and lasted for twenty-four hours. It has recurred several times since then. For six days she has had increasing edema of the feet. Vomiting occurred three days before entrance, at first associated with the anginal pains. For three or four days her cough has grown worse but there has been no sputum.

Physical Examination—On admission the following positive findings were noted. Obesity and slight cyanosis of the mucous membranes. Lungs. Showed slight dulness at both bases posteriorly, with fine moist râles. Heart. Apex impulse was neither seen nor felt. The sounds were regular and rapid (140). There was a thrill at the apex and a blowing murmur over the whole precordium both of which seemed diastolic in time but it was impossible to determine the time accurately because of the rapid heart rate. The murmur was loudest to the left of the sternum, in the fourth and fifth interspaces and was transmitted to the axilla and the back. A very soft systolic murmur was heard at the apex. The pulses were equal, synchronous and of normal volume and tension. The arterial walls were not palpable and the brachials were not tortuous. The blood pressure was 125 systolic and 80 diastolic.

The leukocyte count was 14,000, hemoglobin, 80 per cent, and the smear and differential count were normal. The nonprotein nitrogen was 47.1 mg per 100 cc of blood. The blood Wassermann was negative.

Treatment and Course—The following day the urine showed 71 per cent sugar. Carbohydrate was fed in the form of sugar water and she received an ampoule of digifolin intravenously. The note of that day says "Cyanosis, pallor, blowing murmur at the fourth chondral space probably systolic, second sound not heard, rate rapid. Small amount of pleural fluid. History suggests coronary disease."

On the second day the carbon dioxide tension of the alveolar air was 17. The leukocyte count was 20,000. On the third day the apex rate was 180-190 with a high pulse deficit, apparently auricular fibrillation. The murmur over the precordium was definitely systolic and the sounds were faint. The electrocardiogram taken a short time after this examination showed simply a sino-auricular tachycardia, with a somewhat inverted T wave. One hour

later, the rate was 120 and perfectly regular. Paroxysms of auricular fibrillation were thought quite likely. The respirations were from 35 to 45 per minute. The digitalis was increased. The urine showed 217 per cent sugar and a positive diacetic acid test. The blood sugar was 0.54 per cent. The following day her condition was much worse and she was practically unconscious. She was given orange juice and fat free milk and 400 cc of a 5 per cent sodium bicarbonate solution intravenously. Acetone bodies in the blood were 372 mg per 100 cc. The patient grew rapidly worse and died that evening, the fourth day after admission.

Necropsy — Necropsy 4,116, twelve and one half hours postmortem (abstract). The body is that of a white woman, 52 years of age, 162½ cm long, well developed and stout. Head Not examined. Trunk Slight edema of the feet and ankles. Skin and Mucous Membranes Pale. On section, subcutaneous fat in large amounts. Abdomen and contents essentially negative. Diaphragm Negative. Pleurae Right, a few adhesions to the pericardium and a few posteriorly. Left, a few adhesions posteriorly and one to the diaphragm. Trachea and Bronchi Contain a moderate amount of reddish brown, frothy fluid. The mucosa is brownish red. Lungs The tissue is slightly leathery, salmon colored and yields a considerable amount of thin, brownish red, frothy fluid. Pericardium Negative. Heart The heart weighs 310 gm, a little enlarged. The myocardium, except in an area to be described, is of fairly good consistency, pale, brown red. The right ventricular wall is three to four mm thick, the left ten. The columnae are fairly well marked on the right but flattened on the left. The right cavity is slightly dilated. The left ventricle, antero-laterally to the right and opposite to the interventricular septum in its lower half, presents a pouch like dilatation 8x4½x2½ cm. The cavity on this side was otherwise negative. The auricular appendages are negative. Valve circumferences—mitral 10 cm, aortic 6 cm, tricuspid, 12 cm, pulmonic 8 cm. The aortic cusps show a slight amount of sclerosis. The valves are otherwise negative. Foramen ovale closed. Right coronary artery free, fairly capacious and shows only a slight amount of fibrous sclerosis. Left, first portion clear and shows only a very small amount of fibrous sclerosis but at a point about 3½ cm from its origin the left descending branch is practically occluded by a fibrous plaque. The vessel and its branches beyond this point are very small and dwindle away in the wall of the dilatation previously mentioned. The myocardium in the region of the dilatation is about 3 mm thick and shows a leathery fibrosis. At a point at the base of the dilatation, in the region of the interventricular septum, a few cm above the apical region there is a perforation of the septum about 6x3 mm. The margins are a little irregular, somewhat rounded and the tissue is perhaps a little softer here than elsewhere. On the endocardium of the left ventricle in the region of the dilatation there is a thin, greyish-yellow, weakly adherent layer of thrombotic material. On the right ventricular side of the opening, arising from its margins, there is a rather weakly adherent, frank columnar, thrombotic mass about 2½x2x1½ cm. The mass presents a rather thick, greyish-yellow, granular outer shell with a mottled surface. It surrounds a dirty, brownish to blackish, red, softer material. Aorta The first portion, just above the cusps, shows a small area of arteriosclerosis, elsewhere it is fairly smooth. The ascending and descending portions show a small amount of fibrous sclerosis. The abdominal portion shows a moderate amount of fibrous sclerosis with several thin, small, calcareous plates. The great branches are negative. Pulmonary artery, Veins and Venae Cavae Negative. Liver Negative. Gall bladder Contains about twelve minute, blackish-green concretions. Pancreas Shows general, well marked, fatty infiltration. The pancreatic tissue presents as small, pale, scattered islands in the ocean of fat tissue. Spleen and suprarenals Negative. Kidneys Negative aside from some injection of the vessels. Pelvis, Ureters and Bladder Negative.

Microscopic Examination — Pancreas Fat present Islands few and far between The islands show some hyaline degeneration of their cells Kidneys Acute degeneration Liver Moderate fatty metamorphosis of the liver cells

Bacteriologic Report — Culture of heart's blood on plain agar "Good growth of streptococci"

Anatomic Diagnosis — Diabetes Mellitus, slight arteriosclerosis, arteriosclerotic occlusion of the left anterior coronary artery with an area of chronic interstitial myocarditis and degeneration and perforation of the interventricular septum, mural thrombi of the ventricles, slight hypertrophy and dilatation of the heart, septicemia (streptococcus)

SUMMARY

A case is reported presenting an interesting example of perforation of the interventricular septum of the heart in an unusual location, the heart was normal except for a limited area of sclerosis The patient also showed slight general arteriosclerosis and diabetes mellitus

CLINICAL STUDIES OF THE RESPIRATION VII

THE EFFECT OF GENERAL WEAKNESS AND FATIGUE ON THE VITAL CAPACITY OF THE LUNGS¹

FRANCIS W PEABODY, MD

AND

CYRUS C STURGIS, MD

BOSTON

The clinical significance of spirometry in disease was recognized more than sixty years ago, but in the last decade little attention has been devoted to its practical application. Recently, however, a number of studies on the respiration have again indicated that the character and possible extent of the pulmonary ventilation are intimately related to the vital capacity of the lungs, and that determination of the vital capacity gives important information as to the functional efficiency of the lungs. Clinical observations have shown that the vital capacity of the lungs is of especial interest in pulmonary tuberculosis¹ and in heart disease,² and that in both these conditions it serves as an index of the clinical condition of the patient. As the disease becomes more severe, the vital capacity falls, and as the disease improves, the vital capacity increases and approaches the normal.

In pulmonary tuberculosis and in the other inflammatory or infiltrative lesions of the lung substance, as well as in conditions which, like pleural effusion, interfere with the movements of the lungs, it is quite easy to understand why the vital capacity is reduced. In heart disease the circumstances are often less simple. The tendency to dyspnea on exertion increases progressively as the vital capacity falls, and there is abundant evidence to indicate that the interference with pulmonary ventilation is an important factor in producing dyspnea, but, particularly in the early stages of heart disease, an increased tendency to dyspnea associated with a decrease in vital capacity may be present without any physical signs of involvement of the lungs or pleural

¹From the Medical Clinic of the Peter Bent Brigham Hospital

1 Dryer, G, and Burrell, L S T. *Lancet* 1, 1212, 1920

Wittich, F W, Myers, J A, and Jennings, F L. *J A M A*, 75 1249 (Nov 6) 1920

2 Peabody, F W, and Wentworth, J A. *Arch Int Med* 20 443 (Sept) 1917. McClure, C W, and Peabody, F W. *J A M A* 69 1954 (Dec 8) 1917

cavities A similar condition may also prevail in late stages, for in many cases the extent of the physical signs is not at all in proportion to the great fall in the vital capacity of the lungs Thus a cardiac patient with moderate pleural effusion and atelectasis may have much lower vital capacity than a patient with a tuberculous pleural effusion of the same extent It has been suggested that the limitation of the movements of the lungs in heart disease, of which the low vital capacity is an indication, may be due to changes in the pulmonary circulation, but proof of this is as yet lacking, and the question naturally arises as to whether the low vital capacity may not be due to general muscular weakness Does the low vital capacity in heart disease depend on the fact that cardiac patients are feeble and easily fatigued? If the diminution in the vital capacity is due to general weakness, then its value as a clinical test is distinctly limited, for it merely gives an indication of how feeble or exhausted the patient is If, on the other hand, the fall in vital capacity depends largely on local changes and on the severity or extent of the specific disease, then the test has a much greater clinical significance, for it provides one more method by which the development and retrogression of the disease may be followed The question has an important practical bearing, and it has, therefore, seemed worth while to attempt to determine how great a factor general muscular weakness may be in causing a reduction of the vital capacity of the lungs This problem has been approached by us in two ways In the first place, the vital capacity of the lungs has been determined in a group of patients in whom weakness and a tendency to fatigue was very great, but in whom there was no evidence of disease of the heart or lungs In the second place, patients with heart disease and a low vital capacity have been studied and an attempt made to fatigue the muscles of respiration

1 THE EFFECT OF GENERAL WEAKNESS ON THE VITAL CAPACITY OF THE LUNGS

It has proved to be much more difficult than was anticipated to find suitable cases in which to determine the effect of general weakness on the vital capacity of the lungs This is chiefly due to the fact that all patients with pathologic conditions of the heart or lungs had to be excluded as these lesions in themselves cause a marked decrease in the vital capacity The patients selected were almost all suffering from pernicious anemia, and they are particularly well adapted to the purpose because the outstanding symptoms of the disease are general weakness and a tendency to dyspnea on exertion, while the involvement of lungs and circulation is usually slight One case of typhoid fever is included, the first observation being made on the second afebrile day after a severe course of thirty-five days of fever, at a time when

the patient was extremely weak, and the second observation five weeks later when she had been getting up in a chair for nine days. It was, of course, necessary to choose patients who were willing to cooperate satisfactorily, and with one or two exceptions they are all below 50 years of age. This selection by age was imperative for it is well known that there is normally a decrease in the vital capacity of the lungs after the age of 50. The normal standards on which the percentage of vital capacity is based are those of West,³ according to which the vital capacity is equal to 2.5 liters per square meter surface area in young men, and 2.0 liters per square meter surface in young women. The surface area was determined by the chart of DuBois and DuBois⁴ and depends on height and weight. The observations are shown in Table 1.

2 ATTEMPT TO FATIGUE THE MUSCLES OF RESPIRATION

If the low vital capacity of the lungs in heart disease is due wholly, or in large part, to general weakness or to weakness of the muscles of respiration, it would be natural to expect a fatigue effect to show itself if strain is put on the respiratory muscles. In these observations, the attempt was made to produce such a fatigue effect by taking the vital capacity every 15 seconds for 10 minutes. The method is similar to that employed by Levine and Wilson⁵ in studying patients with "irritable heart." The exertion involved in the test is considerable, and it would not have been surprising to find a gradual decrease in the volume of the maximum expiration as the experiment progressed. This is what was to be expected if weakness and fatigue play an important part in the production of the low vital capacity. As will be seen, no such tendency for the volume of expiration to decrease was observed. Five patients were studied. Four had serious cardiac lesions and vital capacities between 32 and 55 per cent of the normal standards. One patient had pernicious anemia and he was so weak that he could hardly stand on his feet long enough to have his height measured. His vital capacity was 79 per cent of normal. The highest of the first three observations is taken as the vital capacity in each case. The following protocols show the results of these tests of vital capacity.

PROTOCOLS OF OBSERVATIONS

CASE 1—W. J. R., male aged 38 years, height, 165 cm, weight, 68.4 kg, surface area, 1.76 sq m.

Diagnosis—Aortic stenosis and insufficiency, mitral stenosis.

Physical Examination—Patient is sitting propped up in bed and looks dyspneic. Lungs are negative, except for impairment of percussion note at the right base posteriorly below the angle of the scapula, with slight suppression.

³ West, H. F. *Arch Int Med* **25** 306 (March) 1920.

⁴ DuBois, D. and DuBois, E. F. *Arch Int Med* **15** 868 (June) 1915.

⁵ Levine, S. A., and Wilson, F. H. *Heart* **7** 53, 1919.

TABLE 1—DETERMINATION OF VITAL CAPACITY

No	Name	Sex*	Med No	Age	Height in Cm	Weight in Kg	Surface Area in Sq M	Hb %	Erythrocytes	Leuko-cytes	Vital Capacity in Cc	Per Cent of Normal Vital Capacity	Diagnosis	Remarks
1	V A	♀	5485	21	160.5	49.0	1.50	67	2,736,000	4,400	3,500	116	Pernicious anemia	Two months history of weakness
2	M J A	♀	3718	36	157.0	43.0	1.39	35	1,400,000	4,900	2,730	98	Pernicious anemia	Duration of present illness 4 years Chief complaint "weakness," Marked weakness for 2 years
3	P H B	♂	4223	46	169.0	72.0	1.83	25	980,000	1,600	3,650	80	Pernicious anemia	
4	M O	♂	4181	51	160.0	52.0	1.52	43	2,170,000	5,600	3,470	91	Pernicious anemia	Chief complaints "extreme weakness and vomiting," Quit work three weeks before admission on account of weakness and dizziness
5	T J H	♂	3729	17	163.0	44.3	1.45	36	1,192,000	5,850	3,050	84	Anemia, secondary, due to gasoline fumes	
6	E A L	♀	4316	48	162.0	48.3	1.50 ⁷	50	1,480,000	4,200	2,685	90	Pernicious anemia	Onset of present illness one year ago with gradually increasing weakness
7	F A M	♀	4565	38	161.0	46.0	1.46	72	2,520,000	8,000	2,725	94	Pernicious anemia	Four months history of weakness and diarrhea
8	H M S	♀	4478	38	169.0	62.4	1.72	75	3,728,000	10,200	2,550	74	Pernicious anemia	Unable to work for past three months on account of weakness
9	J T S	♂	3863	43	169.0	75.6	1.87	61	2,440,000	4,800	4,550	97	Pernicious anemia	Duration of present illness five months Quit work three weeks ago on account of weakness
10	J S D	♂	14764	40	168.0	63.4	1.72	45	1,072,000	2,300	3,300	77	Pernicious anemia	Could hardly stand on account of weakness
11	A H	♂	15262	42	175.0	51.0	1.60	45	1,400,000	1,200	3,150	79	Pernicious anemia	Duration of present illness twenty months, history of weakness, diarrhea, not able to do even light work for one month
12	R C	♀	15018	46	151.0	43.2	1.34				2,300	86	Typhoid fever	Test made on thirty sixth day of typhoid fever, second day of normal temperature Could not register grip testing apparatus with either hand
13	A P T	♂	15642	47	171.0	53.6	1.62	35	1,824,000	4,800	4,200	104	Pernicious anemia	Weakness and ease of fatigue chief complaints

* In this column, ♂ denotes male, and ♀ female

of breath sounds and diminished vocal fremitus over this area. A few scattered râles are present at both bases behind. There is edema of the lower extremities.

Vital Capacity 1,400 c c, or 32 per cent of normal

Vital capacity test every 15 seconds

1300, 1400, 1200, 1300, 1350, 1350, 1450, 1400, 1400, 1400,
1400, 1400, 1400, 1350, 1400, 1400, 1475, 1475, 1500, 1450,
1475, 1450, 1400, 1450, 1400, 1500, 1475, 1500, 1450, 1575,
1550, 1500, 1500, 1500, 1500, 1550, 1550, 1550, 1550, 1500

CASE 2—A J C, male, aged 50 years, height, 175 cm, weight, 73 kg, surface area, 1.88 sq m

Diagnosis—Chronic myocarditis. Patient is comfortable while lying in bed but gets short of breath walking on level or upstairs.

Physical Examination—Heart is much enlarged and there is a systolic murmur at the apex. There are a few râles at the bases of both lungs, and slight dulness with diminished intensity of breath sounds at extreme base on right.

Vital Capacity 2,400 c c, or 51 per cent of normal (Six months ago, while he was still able to work, his vital capacity was 3,600 c c.)

Vital capacity tests every 15 seconds

2350, 2400, 2300, 2100, 2300, 2600, 2700, 2650, 2700, 2600,
2650, 2550, 2400, 2450, 2400, 2450, 2550, 2400, 2375, 2650,
2700, 2700, 2100, 2500, 2450, 2650, 2300, 2600, 2500, 2600,
2500, 2350, 2400, 2600, 2450, 2600, 2400, 2600, 2600, 2450

CASE 3—A G, female, aged 54 years, height, 152 cm, weight, 80.4 kg, surface area, 1.77 sq m

Diagnosis—Chronic myocarditis. The patient entered the hospital with cardiac decompensation and auricular fibrillation. She is now much improved and the heart is regular. She has just begun to get out of bed.

Physical Examination—Heart is much enlarged, and there is a systolic murmur at the apex. Examination of the lungs is absolutely negative.

Vital Capacity 1,500 c c, or 42 per cent of normal

Vital capacity tests every 15 seconds

1200, 1500, 1350, 1450, 1250, 1500, 1350, 1300, 1600, 1400,
1500, 1600, 1300, 1550, 1600, 1300, 1475, 1600, 1700, 1650,
1750, 1600, 1400, 1475, 1600, 1650, 1550, 1800, 1300, 1800,
1700, 1800, 1700, 1600, 1600, 1300, 1750, 1600, 1650, 1900

The subject tried very hard and always made a prolonged expiration. This made the intervals between tests very short. Twice she was bothered by cough and the intervals after the fifth and thirty-second tests were 30 seconds.

CASE 4—L D, female, aged 58 years, height, 159 cm, weight, 72 kg, surface area, 1.73 sq m

Diagnosis—Chronic myocarditis. Patient entered the hospital badly decompensated and has improved under digitalis. She is still in bed constantly.

Physical Examination—Heart is much enlarged, with a systolic murmur at the apex. Lungs are entirely negative, except for scattered râles at both bases posteriorly.

Vital Capacity 1,900 c c, or 55 per cent of normal

Vital capacity tests every 15 seconds

1900, 1900, 1800, 1850, 1700, 1700, 1800, 1900, 1450, 1850,
1900, 1800, 1900, 1900, 1900, 2000, 1850, 1900, 1950, 1850,
1850, 1880, 1800, 1800, 1900, 1889, 1820, 1840, 1800, 1900,
1900, 1800, 1900, 1850, 1820, 1800, 1600, 1800, 1700, 2050

Expiration was always much prolonged and on two occasions the intervals amounted to 30 seconds.

CASE 5—J S D, male, aged 40 years, height, 168 cm, weight, 63.4 kg, surface area, 1.7 sq m

Diagnosis—Pernicious anemia Patient has been sick a long time and was so weak that he could hardly stand to be measured Hemoglobin, 35 per cent, erythrocytes, 1,073,000, leukocytes, 2,300

Physical Examination—Heart and lungs are negative

Vital Capacity 3,300 c.c., or 78 per cent of normal

Vital capacity tests every 15 seconds

3150, 3300, 3300, 3200, 3200, 3250, 3200, 3200, 3100, 3200,
3100, 3150, 3200, 3100, 3000, 3200, 3300, 3200, 3300, 3400,
3300, 3400, 3450, 3300, 3400, 3450, 3300, 3300, 3400, 3400,
3450, 3400, 3400, 3300, 3350, 3400, 3300, 3400, 3400, 3400

An attempt was made to obtain some quantitative conception of the actual strength of patients by means of testing the strength of the grip of the hands and to correlate this with the vital capacity The method is, of course, very unsatisfactory but the relative results as given in Table 2 are of interest They indicate that there is no relation between muscular strength as tested by this method and the vital capacity of the lungs

TABLE 2—MEASUREMENTS OF STRENGTH OF PATIENTS

Patient	Sex*	Grip		Vital Capacity, C c	Vital Capacity, Per Cent of Normal	Diagnosis
		Right	Left			
A J C	♂	91	81	2,400	51	Cardiac
W J R	♂	80	78	1,100	32	Cardiac
A G	♂	30	25	1,500	42	Cardiac
L D	♂	25	20	1,900	55	Cardiac
A H	♂	60	50	3,150	79	Pernicious anemia
J S D†	♂	66	45	3,300	78	Pernicious anemia
R C‡	♀	0	0	2,300	86	Typhoid fever

* In this column, ♂ indicates male, and ♀ female

† It is perhaps, noteworthy that J S D, the strength of whose grip was fairly good, was so weak that he could hardly stand

‡ The patient had such extreme weakness that she was unable to register even the slightest change on the machine used to test the grip

DISCUSSION

Observations on the vital capacity of the lungs in patients with extreme general weakness but without definite disease of the heart or lungs and without other local cause which would affect the pulmonary ventilation showed surprisingly little variation from the normal In the series of cases reported, the vital capacity was in no instance less than 74 per cent of the normal standards based on body surface area In connection with this, it must be remembered that most normal persons have a vital capacity of at least 90 per cent of the normal standards, but that in a small number (about 5 per cent) of healthy young adults the vital capacity has been found to be between 85 and 90 per cent of the normal On this basis then, one may say that extreme general weakness may account for a decrease in the vital capacity of the lungs

of, roughly, from 20 to 30 per cent below the normal standards. The number of patients examined is small, however, and exceptional cases may show a somewhat greater decrease in vital capacity due to weakness. As patients with severe heart disease may have a vital capacity that is 75 per cent below the normal² it is obvious that general weakness is not a significant factor in accounting for this decrease. Further evidence against the suggestion that fatigue and general weakness play an important rôle in reducing the vital capacity of the lungs in heart disease is the fact that in patients with a low vital capacity the taking of many observations of the vital capacity at short intervals does not result in a falling off of the volume of the respiration as might occur in fatigue.

Another possible factor which might be considered as explaining the inability to breathe deeply in heart disease would be an abnormality of the Hering-Breuer reflex so that the stimulus to expiration or inspiration came prematurely and before the other respiratory phase was complete. Against this hypothesis is the fact that even with severe cardiac disease it is usually possible for patients to hold the breath a considerable time at the end of either full inspiration or expiration. The impulse to take up the other phase of respiration is not extremely pressing. Indeed, the deep expiration is often very prolonged. Most patients with heart disease are willing to cooperate completely in the attempt to determine the vital capacity of the lungs. The result, therefore, as anyone who has had experience will admit, represents the "real" vital capacity and the low value is not due to the fact that the patient is "not trying hard." It is usually simple to pick out the patient who will not cooperate and give what is actually his maximum expiration, and in such case the results must, of course, be discarded as worthless. The evidence here presented supports the conception that the decrease in the vital capacity of the lungs in heart disease is due to a local cause, and is not the result of general weakness. This is of importance for the cause of the decrease, while not as yet wholly clear, is probably intimately associated with the cardiac condition. If the low vital capacity depends on the condition of the heart and circulation and not merely on general weakness, then the determination of the vital capacity has a greater specific significance than would otherwise be the case.

A discussion of the effect of general physical condition on the vital capacity of the lungs would be incomplete without reference to the recent observations made on the vital capacity in cases of "irritable heart" or "effort syndrome." It is characteristic for patients classed in these categories to have physical weakness with a marked tendency to fatigue and breathlessness on exertion combined with a strongly

neurasthenic temperament The most complete study on the vital capacity in this condition is that of Levine and Wilson⁵ who examined 131 British soldiers, grouping them according to the class in which they were discharged from the hospital When compared with the normal standards of Peabody and Wentworth² the average vital capacity for each class was as follows Class 1 97 per cent , Class 2 92 per cent , Class 3 90 per cent , "permanently unfit" 87 per cent The only class, therefore, in which the vital capacity fell below the generally accepted normal standards was the last, and, considering the poor general condition of men discharged from the army as "permanently unfit," it is surprising that the vital capacity was not lower This evidence supports the view that general physical condition is not a factor which causes large variations from the normal in the vital capacity The work of Adams and Sturgis,⁶ who studied the vital capacity in 100 American soldiers with "irritable heart," shows similar results, for only seven men had a vital capacity of less than 85 per cent of the normal Tests of muscular strength showed no relation between the muscular development and the vital capacity Observations of great interest were also made by White⁷ on patients with "effort syndrome" and on neurotic patients with "shell shock" The average vital capacity of his normals was 4,720 c c , of five patients with "effort syndrome," 3,250 c c , and of five patients with "shell shock," 2,240 c c The same relative inefficiency of the "effort syndrome" and neurotic groups was found in applying other respiratory tests such as holding the breath, measuring the expiratory force by means of a manometer, and the "blow-bottle" test White concludes that the vital capacity proved to be rather a test "of nervous stability than of the condition of the cardiovascular or respiratory systems per se, in the groups under discussion" There can be no question as to the correctness of this conclusion, and of the certainty that these groups of men would fail in any test which involved concentration, persistence, or vigorous action The asthenic state is characteristic of the soldier with "shell shock" or "effort syndrome" but the underlying factor is weakness of will and not weakness of muscle The same thing is, of course, true of the neurasthenic of civil life Where the will is weak the vital capacity of the lungs may apparently be low, but the observation will have no value as indicating disease of the heart or lungs because it does not represent a true value Such patients will not exert themselves enough to give what is actually their maximum respiration Levine and Wilson's experience in testing the effect of fatigue on the

6 Adams, F D , and Sturgis, C C Am J M Sc 158 816, 1919

7 White, P D Am J M Sc 159 866, 1920

vital capacity in patients with "irritable heart" is interesting in this connection. They found that immediately after exercise the vital capacity in normal subjects was essentially the same as before exercise, while in soldiers with "effort syndrome" there was a decrease, which in the most severe cases amounted to 18 per cent. Likewise, in the patients with severe "effort syndrome" if vital capacity determinations were made in rapid succession so that 30 readings were made in 10 minutes there was a progressive fall in the volume of the expirations which in some instances amounted to 25 to 50 per cent. This is in direct contrast to our patient with pernicious anemia (No 5) who was so weak that he could hardly stand but who was able to keep up the volume of his vital capacity when forty observations were made in 10 minutes. The striking feature was that in spite of great general muscular weakness the respiratory muscles seemed to maintain a fairly good strength. The will of this patient was strong, his cooperation was good and he was willing to exert himself to his utmost so that the situation was quite different from that with patients with "effort syndrome." Muscular weakness, therefore, has comparatively little effect on the vital capacity of the lungs, but weakness of the will or failure to cooperate and give the maximum expiration are factors which render observations on the vital capacity worthless and misleading. Fortunately, these difficulties are not encountered very frequently in patients with organic disease, and are usually easily recognized when present.

In contrast to the effects of muscular weakness on the vital capacity it is of interest to recall some facts with regard to the effect of physical training. Among eighty-five normal young men whom West³ studied in his attempt to establish normal standards for vital capacity there were twenty-five who gave a history of an unusual amount of athletics and physical exercise. Only two of these had a vital capacity below the average normal standard, in ten the vital capacity was not more than 10 per cent above normal; in eight it was between 10 and 20 per cent above normal, and in five it was between 20 and 30 per cent above normal. The general range of increase of vital capacity above the normal standards associated with the best physical training is thus approximately the same in amount as the decrease below the standards due to extreme physical weakness.

CONCLUSIONS

- 1 The vital capacity of the lungs in patients with great physical weakness, but without disease of the heart or lungs, was found to be not more than 26 per cent below the normal standards. In heart disease, the vital capacity may be as much as 75 per cent below the normal standards.

2 Repeated tests of the vital capacity were made every 15 seconds for 10 minutes in patients with severe heart disease. The exertion involved was considerable, but no evidence of fatigue of the muscles of respiration was observed. The volume of the maximum expiration was as great at the end of the series of tests as at the beginning.

3 These observations indicate that general muscular weakness and fatigue of the muscles of respiration are not important factors in causing the reduction of the vital capacity of the lungs in heart disease.

STUDIES ON ERYTHROCYTES, WITH SPECIAL REFERENCE TO RETICULUM, POLYCHROMATO- PHILIA AND MITOCHONDRIA*

J ALBERT KEY, M D

BOSTON

INTRODUCTION

It has been suggested (Shipley,¹ Sappington²) that the study of the mitochondria in the erythrocytes in the circulating blood would be of value clinically in estimating the state of activity of the bone marrow

The term mitochondria was introduced by Benda³ in 1899 to designate certain threadlike granules which he had noted in his studies on spermatogenesis Many authors had previously described similar protoplasmic granules under various terms The protoplasmic fila of Flemming⁴ included some poorly fixed mitochondria, and a large percentage of the bioblasts of Altmann⁵ were mitochondria Cowdry⁶ has carefully reviewed a very extensive literature on the subject and critically examined the descriptions of protoplasmic granules as given by a great many investigators under a very varied terminology He finds that granules having definite characteristics and hence capable of being separated from other cytoplasmic constituents have been observed and described in the protoplasm of almost every type of animal and plant cell He suggests that in order to avoid confusion in the literature, the term mitochondria be used to designate these granules

Altmann regarded his bioblasts as being the ultimate divisions of life Different investigators have credited mitochondria with performing a great variety of functions, such as transmission of hereditary characteristics, formation, directly or indirectly, of collagenic fibrils,

*This is study No 9 of a series of studies of the physiology and pathology of the blood from the Harvard Medical School and allied hospitals This study was made under a grant from the Proctor Fund of Harvard University for the Study of Chronic Diseases

*From the laboratory of the Department of Medicine of the Harvard Medical School

1 Shipley, P G The Mitochondrial Substance in the Erythrocytes of the Embryo Pig, *Fol Haematol* **20** 61, 1915

2 Sappington, C O The Occurrence of Mitochondria in the Red Blood Corpuscles During Experimental Anemia, *Arch Int Med* **21** 695 (May) 1918

3 Benda, C Weitere Mitteilungen uber die Mitochondria, *Verhandl d Physiol Gesellsch*, 376, 1899

4 Flemming, W Zellsubstanz, Kern und Zellteilung, Leipzig, 1882 (quoted from Cowdry)

5 Altmann, R Die Elementarorganismen und ihre Beziehungen zu den Zellen Leipzig, 1890

6 Cowdry, E V The Mitochondrial Constituents of Protoplasm Contributions to Embryology No 25 Publication Carnegie Inst No 271 1918, p 41

plant plastids, myofibrils, epidermis fibrils, neurofibrils, pigments, zymogen granules of pancreas, and secretion of other glandular cells, etc. Other investigators believe that they have some function related to the general metabolism of the cell or connected with the respiration of the cell. In spite of the large amount of careful work on the subject, the function of the mitochondria is unknown. The literature is fully reviewed by Cowdry.⁶

The granules which can be classed as mitochondria have certain characteristics which can be enumerated, briefly, as follows: (1) They are cytoplasmic structures. No constant relation to the nucleus has ever been demonstrated. (2) They have a higher degree of refraction than the surrounding cytoplasm and unless obscured by granules or other cytoplasmic inclusions can be seen unstained in the living cell. (3) In morphology they vary from small granules or short rods to long threadlike granules or rods. They may be seen to change form while under observation and to move slowly about in the cytoplasm (Lewis and Lewis⁷). They are discrete bodies free in the cytoplasm and while the long threadlike forms may be entangled to resemble a net they do not form a continuous reticulum. (4) They disappear from the cells a few hours after the death of the animal. It is usually stated, that in order to demonstrate the mitochondria, the tissue must be fixed within two hours after death. However, I have been able to stain mitochondria in thyroid tissue from a necropsy performed six hours after death and in nerve cells from spinal ganglia of rats which were fixed twenty-four hours after the animal was killed. The rapidity with which they disappear, apparently varies with the speed of autolysis within the given cell. (5) They disappear quickly in cells heated to from 48 to 50 C. Thirty minutes is the time given by Cowdry.⁸ (6) Chemically mitochondria are believed to be a combination of phospholipin and albumin. They are soluble in dilute 2 per cent acetic acid and in the various mineral acids. They are soluble in alcohol, ether and chloroform, and are rendered insoluble by chromization. They do not stain with Sudan III or Scharlach R, and may be colored brown by osmic acid but are not blackened as are neutral fats. They do not give a definitely positive Millon's reaction.

Mitochondria are best fixed by mixtures of liquor formaldehyd and potassium bichromate, osmic acid, potassium bichromate and a low percentage of acetic acid, or osmic acid, chromic acid and a low percentage of acetic acid, or osmic vapor where it can be applied to

⁷ Lewis M R and W H. Mitochondria (and other cytoplasmic structures) in *Tissue Cultures*, *Am J Anat* **17** 339, 1915.

⁸ Cowdry, N H. A Comparison of Mitochondria in Plant and Animal Cells, *Biol Bull* **33** 196, 1917.

smears In sections of fixed tissue they are best stained by Altmann's anilin-acid-fuchsin differentiated with methyl green or picric acid, Regaud's iron alum hematoxylin method, or Benda's crystal violet-alizarin method By all methods, the sections are stained deeply and then differentiated to bring out the mitochondria A number of proven technics are given in Cowdry's ⁶ article

Janus green B (diethylsafraninazodimethylanilin) of Farbwerke Hoechst and Co, used as a supravital stain in dilute solutions of from 1 5,000 to 1 100,000 in 0.85 per cent sodium chlorid solution, stains mitochondria an intense green, and unless the dye is too concentrated no other cytoplasmic structures are stained In properly fixed and stained preparations the morphology and distribution of the mitochondria correspond to their morphology and distribution in the same type of cells when examined unstained or stained supravitaly with Janus green B

Mitochondria were first noted in red blood cells by Meves ⁹ in 1907, who briefly described them as a perinuclear mass of threadlike granules which outlast the nucleus and are found in young erythrocytes In 1911, Ciaccio ¹⁰ found the mitochondria gradually decreased as the hemoglobin appeared in the cell and concluded that they had to do indirectly with the formation of hemoglobin He described a few mitochondria as being present in erythrocytes just before they entered the circulation Meves, ¹¹ in 1911, made practically the same observation on red blood cells in the bone marrow of the guinea-pig Schridde, ¹² in 1912, studied bone marrow of young rabbits by Altmann's technic and claimed that the mitochondria lose their sharp contour, shrink in size, and diminish in number in direct proportion to the increase in hemoglobin in the cell From these observations, he concluded that the mitochondria are the first elements in hemoglobin formation, and are able to fill the whole cell with a chemical element (hemoglobin)

Ciaccio ¹³ immediately repeated his former work using the technic of Altmann, Regaud, Benda and Ciaccio, and concluded that the claims of Schridde were without foundation because his technic was inadequate—the objection being that the acid fuchsin used by Schridde to stain the mitochondria colors the hemoglobin vividly and thus obscures

9 Meves, F Ueber Mitochondrien bezw Chondriokonten in den Zellen junger Embryonen, *Anat Anzeig* **31** 399, 1907

10 Ciaccio, C Sui mitochondri degli elementi linfoidi e mieloidi *Pathologica* **3** 1911 (quoted from Cowdry)

11 Meves, F Gesammelte Studien an den roten Blutkörperchen der Amphibien *Arch f mik Anat* **77** 465, 1911

12 Schridde, H Untersuchungen ueber die Bildung des Haemoglobins *Anat Anzeig* **42** No 20-21, 1912

13 Ciaccio, C Les plastosomes des éléments de la série hémoglobinique *Fol Haematol* **15** 391, 1913

the mitochondria in the cells containing hemoglobin He, therefore, holds to his original view that the mitochondria play an indirect part in the formation of hemoglobin Cowdry⁶ notes that Ciaccio ignores the fact that the iron-hematoxylin stain which he himself used, acts in much the same way, and recommends supravital staining with Janus green B as a method by which mitochondria can be studied and counted in living blood cells He also states that the formation of hemaglobin from mitochondria alone involves chemical impossibilities

Cowdry,¹⁴ in 1914, stained the bone marrow of a young guinea-pig with Janus green B and noted that mitochondria were present in some of the erythrocytes Shipley,¹ in 1916, studied the mitochondria in the erythrocytes of embryo pigs and found them present in all nucleated red blood cells and in a percentage of the erythrocytes which decreased as the age of the embryo advanced (73 per cent in 24 mm embryo, and 12 per cent in 100 mm embryo) In fresh unstained blood the mitochondria could be seen by transmitted light as refractile granules and by reflected light as bright granules surrounded by a bright halo They were stained specifically with Janus green B and gave all of the staining reactions of mitochondria Their solubility in acetic acid was tested by fixing blood smears in Bensley's acetic-osmic-bichromate mixture containing varying amounts of acetic acid In fluid containing more than 3 drops of acetic acid in 20 c c, the mitochondria were not fixed, and in fluid containing more than 10 drops of acetic acid in 20 c c, the red blood cells were destroyed

In fresh blood stained supravitaly with Janus green B (1 10,000 in 0.85 per cent sodium chlorid solution), the mitochondria were described as dancing green rods which moved about so rapidly in the cell that it was difficult to count them They exhibited a tendency to assume a position perpendicular to the slide and appear as granules unless the cell was turned on edge They were often clumped together near the periphery of the cell After about two and one-half hours, the bright green color was changed to a red from the reduction of the dye to diethyl-saffranin This faded slowly They were also described as forming green walled vacuoles which might be extruded or fragmented The mitochondria disappeared from all cells in shed blood after a short time They were not present in the erythrocytes of the circulating blood of normal adult mammals, but were present in the young erythrocytes of normal adult bone marrow and in the circulating blood of adult humans with pernicious anemia and signs of active blood regeneration Their presence in erythrocytes was evidence of the youth of the cells containing them

14 Cowdry, E. V. The Vital Staining of Mitochondria in Human Blood Cells with Janus Green B. *Internat Monatschr f Anat u Physiol* **31** 267, 1914

Sappington,² in 1918, using the Janus green B stain (1 6,000 in 85 per cent sodium chlorid solution), studied the blood of rabbits which had been rendered anemic by repeated bleedings or by repeated injections of phenylhydrazine hydrochlorid. He found that the number of erythrocytes containing mitochondria increased as the anemia became more severe, and that the percentage was inversely proportional to the percentage of hemoglobin in the blood. In the animals which had been rendered anemic by phenylhydrazine, the mitochondria of the erythrocytes were slightly larger and more deeply stained than those in the animals which had been bled. Otherwise, the conditions were the same in the two series. He was able to produce such a severe anemia that 80 per cent of the erythrocytes contained mitochondria, whereas none were seen in the blood of normal rabbits. The mitochondria were described as bright green dancing granules. Both Shipley and Sappington concluded that the study of the mitochondria in the erythrocytes in anemia would be a valuable clinical method for the detection of blood regeneration.

It has long been known that a basic staining substance is present in certain erythrocytes, and that it can be demonstrated in the form of polychromatophilia, punctate basophilia, or reticulum by appropriate staining methods. In dried or fixed smears stained with Wright's modification of Romanowsky's stain polychromatophilia is seen as a diffuse double staining of certain erythrocytes with blue and red which gives them a light purplish color. By the same method, punctate basophilia or stippling is seen as small deep blue granules in certain erythrocytes. The reticulum present in certain erythrocytes is seen as a delicate network and is best demonstrated by staining supravitaly with brilliant cresyl blue or azur II but can be stained supravitaly by almost any basic dye which mixes with the blood without forming a precipitate and enters the erythrocytes as azur I, methyl, gentian or crystal violet, pyronin, malachite green, thionin, neutral red, the various methylene blues, etc.

It is quite generally believed that these basophilic forms are young erythrocytes. The reasons for this belief are, briefly, as follows: in the circulating blood of normal adult humans no polychromatophilia or punctate basophilia and not over 1 per cent of reticulated cells are found. In the blood of mammalian embryos, the basophilic erythrocytes occur frequently and their percentage decreases as the age of the embryo advances. Shortly after birth, the erythrocytes of the circulating blood resemble those of the adult. Basophilic substance can usually be demonstrated in nucleated red blood cells. In adult bone marrow, where blood formation is in progress, basophilic erythrocytes are common. In anemic adults the percentage of basophilic erythrocytes

is roughly proportional to the activity of the bone marrow. Finally, Robertson,¹⁵ in 1917, by transfusing normal rabbits (giving them 10 c c of whole blood daily for from 10 to 20 days) was able to increase their hemoglobin from 80 or 90 per cent to 140 or 150 per cent. In these transfused rabbits with an increased percentage of hemoglobin, the reticulated cells in the circulating blood fell from 10 or 20 per 1,000 erythrocytes in the normal animal to about 1 per 1,000 in the transfused animals, and in the bone marrow from 320 per 1,000 to 14 per 1,000. The theory that basophilic erythrocytes are old senile erythrocytes is no longer tenable.

Hawes,¹⁶ in 1909, investigated the three forms of basophilia exhibited by erythrocytes. He made careful percentage counts of polychromatophilic and stippled cells in smears stained by Wright's stain and compared them with the percentage of reticulated cells found in specimens of the same blood stained supravitally with brilliant cresyl blue. The counts of reticulated cells were made from permanent smear preparations obtained by staining a drop of blood on a cover slip with brilliant cresyl blue, then smearing the blood and staining the dried smear with Wright's stain in the usual manner. The study included blood from normal adult humans, normal infants, and adult patients with various forms of primary and secondary anemia. Biffi's statement that the polychromatic cells are usually larger than normal was confirmed, and it was noted that reticulated cells were, as a rule, larger than the normal cells. No distinction was made between polychromatophilia and stippling but the total percentage of both forms present was compared to the percentage of reticulated cells in the same blood. He found that the two groups were roughly proportional for the same blood but that the percentage of reticulated cells was uniformly higher and concluded that they were different forms of the same process. The supravital staining method was a more delicate method and hence gave a slightly higher percentage.

Pappenheim¹⁷ studied the basic substance in young erythrocytes by means of his analytical staining methods. He used various combinations of methyl green, pyronin, methylene blue, eosin, malachite green, azur II, giemsa, triacid and gold orange. On the basis of their staining characteristics, he concluded that polychromatophilia, punctate basophilia and reticulum were all of spongioplasmatic nature and not

15 Robertson, O. H. Effects of Experimental Plethora on Blood Production. *J. Exper. M.* **26** 221, 1917.

16 Hawes, J. B. A Study of Reticulated Red Blood Corpuscles by Means of Vital Staining. Its Relation to Polychromatophilia and Stippling. *Boston M. & S. Jour.* **161** 493, 1909.

17 Pappenheim, A. Neue Zytomorphologische Studien an Blutzellen mit farben analytischen Methoden. *Fol. Haematol.* **9** 572, 1910.

paraplasmatic, chromatic or parachromatic, the spongioplasm being the indifferent cytotreticulum and the paraplasma the true protoplasmic parenchyma of the cell. The Howell-Jolly bodies stained with methyl green and hence he concluded that they were of chromatic nature (nuclear rests). In his general review of the subject,¹⁸ he regards the Cabot rings as rests of the nuclear membrane and the metachromatic granules of the reticulum which stain red with brilliant cresyl blue or azur II as lipoid material because they are soluble in water. He also notes that punctate basophilia is characteristic of toxic regeneration and regards it as a further modification of polychromazie than is reticular substance.

Shilling-Torgau,¹⁹ in two beautifully illustrated articles, reviewed an extensive literature and summed up his own studies on the erythrocytes. He concluded that in the hemoglobin containing part of the mammalian erythrocyte, apart from the glass body and capsule body, there is an achromatic ground substance or stroma of unknown structure which condenses on an endoplasm boundary to form a crust, apparently through the medium of cholesterol and lecithin inclosures, while the inner part can be more fluid. In the young erythrocytes the basic substances are contained in those layers which through pictures of polychromatophilia, punctate basophilia and vital staining net structure deviate from the ground substance. They are due to the presence of basic staining protoplasm of youthful character and are all different expressions of what is substantially the same substance. Punctate basophilia is a modification of the young basic protoplasm dependent on embryonic or pathologic disturbances of blood building. The distributed metachromatic vital substance apparently takes origin from the region of the "Kapselkorper" or, at least, from a small eccentric district in the vicinity of the "zentral apparates". The blood platelets arise from the nucleus and are eliminated by extrusion.²⁰

It is seen that the red blood cells described by one group of investigators as containing mitochondria correspond closely to those described by another group of investigators as containing reticulum or other forms of basophilic substance. In fact, with the exception of Meves¹¹ none of the investigators quoted as describing mitochondria in red blood cells have even mentioned the reticulum. Meves in his studies on amphibian red blood cells laid stress on the "randriefen" or band

18 Pappenheim, A. Ueber Polychromatophilie. Synthetische Generalübersicht. *Fol. Haematol.* **9** 311, 1910.

19 Schilling-Torgau. Arbeiten über den Erythrocyten. *Fol. Haematol.* **11** 572, 1911, **14** 97, 1913.

20 Wright's theory of the origin of the platelets from the megakaryocytes is accepted in this country, and I have confirmed his results in unpublished observations. (*J. Morphol.* **21** 263, 1910.)

around the periphery of the cell, the chondriosomes (mitochondria), and the chromatoiden granules around the nucleus. He dismissed the basophilic network or reticulum as an artefact and quoted A. Fischer²¹ to the effect that the chief part of the red blood cell—the hemoglobin, from a neutral solution through the action of various fixatives becomes insoluble and takes on a plasmatic appearance. The coagulations are fine with osmic, Altmann's, picric, chromic, osmic acetic, platonic chlorid, formol, etc., and coarse with nitric acid or nitric acid alcohol.

As no attempt has been made to differentiate the two structures, many men interested in hematology have assumed that the reticulum is a form of mitochondrial substance.

MATERIAL AND METHODS

Rabbits were used because they are convenient to work with and because mitochondria and reticulum have been described in the young erythrocytes of rabbit's blood. As no quantitative data was sought, the adult rabbits were simply bled from an ear vein often enough to keep them moderately anemic and stimulate the bone marrow sufficiently to produce from 10 to 15 per cent of reticulated cells in the circulating blood. Bleedings of from 10 to 20 c.c. of blood, every second or third day, were found to be satisfactory. At times, the degree of anemia was increased in an effort to study earlier stages in blood formation. Other rabbits were rendered anemic by the injection of phenylhydrazin hydrochlorid. As a rule, the blood was shed into a bottle containing a few crystals of potassium oxalate and could then be kept and studied for days. All observations on oxylated blood were controlled by the study of fresh blood. The oxalate apparently did not affect either the reticulum of the erythrocytes or the mitochondria of the white blood cells. In other instances, the blood was defibrinated by stirring rapidly with a glass rod. This process markedly reduced the percentage of white blood cells in the blood, but did not so greatly reduce the percentage of reticulated cells. Blood of embryo cats and embryo rabbits was also studied.

Fresh unstained blood was examined by transmitted light and with the dark field apparatus. Blood was stained supravitaly with brilliant cresyl blue, azur I, azur II, various methylene blues, methyl green, gentian violet, crystal violet, thionin, malachite green, neutral red, and Janus green B. The Janus green B used was the diethylsafranin-azodimethylanilin of Farbwerke Hoechst and Company and was given me by Professor R. R. Bensley of Chicago. It stained undoubted mitochondria in white blood cells and in gland cells.

21 *Fixierung, Färbung, und Bau des Protoplasmes*, Jena, 1899.

Attempts were made to control the observations on fresh preparations. The mitochondrial fixatives of Bensley,²² Meves,²³ and Regaud²⁴ were used, also various modifications of these were tried. Dried and wet smears were fixed in osmic and liquor formaldehyd vapor and ethyl and methyl alcohol and in various mixtures containing liquor formaldehyd potassium bichromate, corrosive sublimate, acetic, picric or chromic acid. Fixation of whole and partially laked blood was accomplished by mixing the blood with the fixing fluid, then washing and collecting the sediment. The fixed cells were examined unstained and smeared on a slide and the dried smear stained. In other instances the sediment was dehydrated in graded alcohol cleared in xylol, collected in a filter paper cone and imbedded in paraffin. The paper was removed and 2 micron sections were cut and fixed to slide by water-albumin method and stained for mitochondria. Bone marrow of anemic rabbits and livers of embryo cats were fixed by Bensley's A O B, Regaud's IV B or Meves method, and sections cut and stained for mitochondria.

The mitochondrial stains of Bensley, Regaud and Benda²⁵ were used. Smears and sections were also stained with the various dyes which stain reticulum supravitaly and by the common blood stains. The method of Hawes¹⁶ and of Cunningham²⁶ of staining supravitaly and then making a smear and staining the dried smear was also used.

OBSERVATIONS

If fresh blood from an anemic rabbit be examined by transmitted light with an immersion lens, the erythrocytes appear homogeneous. However, if the cover slip be pressed down so that the cells are actually flattened out between the slide and the cover slip and the condenser is slightly lowered, one can occasionally see an indefinite faintly refractile substance in some of the erythrocytes. The picture is very indefinite and not to be compared in clearness with the refractile mitochondria seen in the lymphocytes in the same preparation. The cells exhibiting this refractile substance are usually slightly larger and lighter in color than the surrounding erythrocytes.

22 Bensley, R. R. Studies on the Pancreas of the Guinea Pig. *Am J Anat* **12** 297, 1911.

23 Meves, F. Die Chondriosomen als Trager erblicher Anlagen. *Cytologische Studien an Huhnen Embryo*. *Arch f Mik Anat* **72** 816, 1908.

24 Regaud, C. Etude sur la structure des tubes seminiferes, etc. *Arch d'Anat Microp* **11** 291, 1910.

25 Benda, C. Die Mitochondriafarbung und andere Methoden zur Untersuchung der Zellsubstanzen. *Verhandl d Anat Gesellsch*, 1901, p 155.

26 Cunningham, T. D. A Method for the Permanent Staining of Reticulated Cells. *Arch Int Med* **26** 405 (Sept) 1919.

If the blood be mixed with 0.85 per cent sodium chlorid solution and examined by reflected light with the dark field condenser, the normal erythrocytes appear as luminous rings with a uniformly dark interior. In blood containing from 15 to 20 per cent of reticulated cells no difference could be made out between the various erythrocytes unless the cells were laked or stained supravitaly when the reticulum became visible as luminous masses in the cells containing it.

When a drop of blood is mixed with a drop of brilliant cresyl blue (1:300 in 0.85 per cent sodium chlorid solution) the reticulum is quickly stained a deep purple. In anemic rabbits' blood and in embryonic blood, the reticulum is usually stained as a delicate mosslike wreath lying in the thick zone of the erythrocyte between the periphery and the central depressed area. The fibers of the wreath are not clear cut or straight but are fuzzy and wavy as though they were made up of minute particles. They branch irregularly and vary in caliber from fairly coarse to extremely delicate fibrillae. In the meshes or at the internodes of the coarser fibers are a variable number of purple staining granules. A few (from two to six) minute spherical metachromatic granules are usually present in the net. These stain red at first and later change to a purple and cannot be distinguished from the rest of the reticular substance.

The mosslike wreath is the usual form, but all gradations, ranging from cells containing a few basophilic granules or fragments through rather coarse networks and loosely constructed wreaths up to the typical wreath can be seen in the same blood. A rather common form is one in which from twelve to fifteen anastomosing fibers form an irregular network in the cell. This irregular form is more common in rabbits rendered anemic with phenylhydrazin and is the usual form seen in pathologic anemias in human blood. The fibers are coarse and the granules larger than in the typical forms. In addition to the cells showing definite basophilic granules or masses, one often sees cells which stain diffusely with the dye and are light purple in color. Drs. Minot and Peabody have noted these cells and term them grey cells.²⁷ Presumably they represent a transition stage between the reticulated cell and the mature erythrocyte. With azur I they stain a diffuse light pink. Attempts to differentiate them more sharply from the normal erythrocytes were not successful.

In the nucleated red blood cells of embryonic blood, the reticular net surrounds the nucleus. In the cells in which the nucleus is beginning to be cast off the reticulum is flattened between the nucleus and the cell membrane. In cells in which the nucleus is about half way out of the cell the encircling reticulum is broken and the tapering edges are seen

27 Personal communication

in the angular space between the partially extruded nucleus and the cell membrane. In cases in which the nucleus has almost entirely left the cell, the typical reticular wreath is formed in the erythrocyte adherent to the nucleus. In preparations apparently perfectly stained, an occasional nucleated red blood cell is found in which no reticular substance can be seen. No evidence was seen which suggested that any of the basophilic substance left the cell with the nucleus, as one would expect to occur if the nucleus were pinched off by a process of unequal cell division as described by Emmel²⁸. The behavior of the reticulum lends support to the theory of Howell²⁹ that, as a rule, the nucleus is actually extruded.

Azur II, used as a supravital stain, stains the reticulum and nuclei blue and the metachromatic granules red. It is a much more reliable stain for the metachromatic granules than the brilliant cresyl blue. Azur I stains the reticulum pink and the nuclei purple. Thionin stains the reticulum pink and the nuclei purple. Toluidin blue and Unna's alkaline methylene blue each stain the reticulum pink and nuclei blue. Crystal violet, gentian violet, methylene blue, Ehrlich's methylene blue, neutral red, malachite green, etc. stain the reticulum and nuclei the color of the dye used and not differentially. Methyl green stains nuclei green but does not stain reticulum.

Because Janus green B has been used extensively as a specific supravital stain for mitochondria, its action was studied more in detail. It was used in concentrations varying from 1:5,000 to 1:100,000, 1:6,000 in 0.75 per cent sodium chlorid solution was found to be most satisfactory for red blood cells. A drop of the stain is placed on a slide, and a very small drop of blood added with a small glass rod and the preparation covered with a cover slip. The preparation is ringed with petrolatum and can be studied at intervals for days. In such a preparation the mitochondria of the lymphocytes are stained a deep green in about two minutes and can be seen as minute rods and granules lying free in the protoplasm. A few minutes later the mitochondria of the granular leukocytes are stained green and can be differentiated from the specific granules which surround them but remain clear and unstained. At about the same time, certain of the erythrocytes are seen to be stained a faint diffuse slate green color and contain a variable number of small green granules and rods which resemble the mitochondria seen in the white blood cells in the same preparation. In an isotonic medium these granules are stationary and

28 Emmel, V. E. Concerning Certain Cytological Characteristics of the Erythroblasts in the Pig Embryo and the Origin of Non-Nucleated erythrocytes by a Process of Cytoplasmic Constriction. *Am J Anat* **16** 127, 1914.

29 Howell, W. H. The Life History of the Formed Elements of the Blood, Especially the Red Blood Corpuscles. *J Morphol* **4** 57, 1890.

exhibit neither slow nor rapid movement. If the blood be brought into a hypotonic medium so that part of the red cells are laked, the granules may move rapidly about in the cell as do the granules of the leukocytes under the same conditions.

In from fifteen to twenty minutes the green granules in the erythrocytes have become more numerous, and are connected by delicate green strands so that a definite reticulum is formed in the erythrocytes. The network is never in the form of the mosslike wreath seen in preparations stained with brilliant cresyl blue or azur, but when fully stained it is an irregular net of rather coarse fibers crossing the cell irregularly and resembles the reticulum seen in pathologic anaemias in human blood. The granules are incorporated in the fibers and lose their identity. At about the same time, the nuclei of the white blood cells are stained greenish blue. In about one-half hour, the nuclei of the white blood cells gradually turn pink in color from the reduction of the green dye to the diethyl-saffranin. In the majority of cases, mitochondria of the white blood cells were not observed to turn pink, as is usually described, but, as a rule, gradually faded, and after from three to six hours they had entirely disappeared. In other cases, they apparently coalesced to form one or two deep green round or oval masses which remained in the cell several hours longer and then disappeared. In other preparations made from the same blood with the same stain, in about the same proportions, the mitochondria turned pink and disappeared in two hours.

The reticulum in the erythrocytes, which was stained a deep green in about one-half hour, neither turned pink nor faded but remained a uniform deep green for from twenty-four to forty-eight hours. In from eight to ten hours, most of the reticulated erythrocytes are laked, and in twenty-four hours all of the reticulated and many of the non-reticulated erythrocytes are laked, and the bright green reticulum can be seen in the shadows, and its morphology does not differ markedly from the morphology of the reticulum in a fresh Janus green preparation made from fresh blood. After twenty-four hours, the green color gradually fades from the reticulum, as does the pink from the nuclei, and in about forty-eight hours the preparation is clear and unstained. The reticulum, however, does not disappear from the cell after the Janus green fades, but remains as a refractile network in the shadows of the laked cells. Its morphology is not altered to any great extent, and it apparently persists as long as the cell membranes remain intact and the preparation is prevented from drying. In well ringed preparations it remains practically unchanged for two weeks or more.

Vacuoles are not rare in erythrocytes of a preparation which has stood for an hour or so. They are clear and variable in size and are usually seen in the nonreticulated cells. When present in reticulated

cells, they apparently have no connection with the substance stained with Janus green. No evidence was seen which suggested that this substance swelled to form vacuoles or globules. No green walled vacuoles were seen, and it is believed that the vacuoles observed here are similar to the degeneration vacuoles seen in other cells. Another form of degeneration occasionally seen in preparations which have dried slightly and very slowly is one in which the cell membrane preserves its smooth contour but the hemoglobin apparently contracts and splits to form two rounded globules in the cell. The globules may be unequal in size and a clear area is seen in the angular space between the globules and the cell membrane. This phenomenon may occur in either reticulated or nonreticulated cells. When it occurs in cells in which the reticulum is stained, the reticulum is in the globules containing the hemoglobin and may be in one or in both of them. Such forms may be quite numerous in a localized area, and the rest of the preparation show only beginning crenation, so they are apparently due to osmotic influences and not to any inherent property in the erythrocytes affected.

In oxydated blood shed twenty-four hours before and kept at room temperature, the reticulum can be stained with Janus green and does not differ in morphology from that stained in fresh blood. In oxydated blood, the mitochondria disappear from the white blood cells in from five to six hours at room temperature and none can be seen in the twenty-four hour blood. If a preparation of blood stained with Janus green be allowed to stain completely, and then 5 per cent acetic acid be run under the cover slip, the mitochondria quickly disappear from the white blood cells and the nuclei become refractile and then slowly fade. The erythrocytes are quickly laked, and the bright green reticulum is seen in the shadows of the laked reticulated cells. Its color fades, as does that of the nuclei, but instead of disappearing entirely from the cell, as do the mitochondria of the lymphocytes, the reticulum becomes refractile and can be seen in the laked erythrocytes. If the preparation be examined twenty-four hours later, the refractile reticulum is still present.

If a small amount of 1:300 solution of azur I be allowed to flow under the cover slip of a preparation in which the reticulum is deeply stained with 1:6,000 solution of Janus green, the green reticulum is quickly stained pink by the azur. When the process of staining is watched in an individual cell, the green apparently does not fade but is covered by the pink dye and obscured. The morphology of the reticulum is unchanged, except that the fibers of the irregular network appear coarser from the addition of the azur. It is thus possible to stain the reticulum as an irregular net with azur by first

staining with Janus green, and in another drop of the same blood by using the azur alone to stain it as a mosslike wreath in the great majority of the cells. As noted above, when stained with Janus green the mosslike wreaths are not formed, and none are seen when the preparation is later stained with the dyes which characteristically give the mosslike wreath. The supravital staining apparently determines the form of the reticulum and fixes it in the form characteristic of the dye used. The percentage of erythrocytes containing reticulum in a preparation stained with Janus green corresponds closely with the percentage of reticulated cells in a preparation of the same blood stained with brilliant cresyl blue, azur I, or II, etc.

Janus green B stains mitochondria in very dilute solutions of the dye. A solution of 1:100,000 in 0.85 per cent sodium chlorid apparently stains all the mitochondria in the cells examined and stains them intensely. Cowdry⁶ was able to stain mitochondria in lymphocytes with solutions of 1:1,500,000 Janus green B in physiologic sodium chlorid solution. In order to stain the reticulum successfully, a higher concentration of the dye is necessary. Solutions of 1:10,000 work fairly well but act slowly. With a solution of 1:20,000 it is necessary to use a larger volume of stain and the action is slow and uncertain. The reticulum, when stained fully, has the characteristic irregular netlike morphology but the structure is more delicate and the percentage of reticulated cells stained in the preparation is less than that in a specimen of the same blood stained with a solution of the same dye 1:6,000. In order to stain the reticulum with a solution of 1:50,000 Janus green B, from three to six drops of blood were mixed with from 6 to 10 c.c. of the dye and the sediment examined several hours later. It was possible to stain a very delicate netlike reticulum in a percentage of the erythrocytes which was well below that of the reticulated cells actually present in the blood examined³⁰. No evidence was obtained which suggested that the reticulum contained any inclusions or granules which possessed highly specific affinity for Janus green and therefore might be of mitochondrial nature. The morphology of the reticulum, when completely stained, was always an irregular net. In using Janus green it is necessary to protect the erythrocytes from crenation as the stain apparently does not enter crenated red blood cells. In a preparation in which the red blood cells are even slightly crenated the reticulum of the erythrocytes is not stained even after many hours, although the mitochondria of the white blood cells are stained perfectly in a few minutes. It was for this reason that the slightly hypotonic saline solutions (from 0.75 to 8 per cent) were used.

30 With solutions of 1:100,000 nothing was stained in any erythrocytes

CHARACTERISTICS OF YOUNG ERYTHROCYTES

Considering erythrocytes which contain basophilic substance as young erythrocytes, it has been noted by Biffi³¹ and confirmed by Hawes,¹⁶ that these cells are, as a rule, slightly larger than the mature cells in the same preparation. In my investigations the majority of the reticulated cells seen were larger than the mature cells. However, I have found many cells of normal or less than normal size and even microcytes which contained reticulum. The larger cells are usually slightly lighter in color than the surrounding cells and apparently contain a lower concentration of hemoglobin. The reticulated cells exhibit a tendency to agglutinate and to adhere to foreign bodies in the blood. Their behavior in this respect resembles that of the leukocytes but is less marked. In oxydated blood which is stained and examined without unduly agitating the cells, frequent clumps of from ten to twenty reticulated cells are seen, even in blood containing only from 5 to 10 per cent of reticulated cells. One or more white cells may be present in the mass of reticulated cells. This tendency to agglutinate is particularly marked when the oxydated blood is in hypotonic salt solution.

The specific gravity of the reticulated cells is slightly lower than that of the normal erythrocytes. In a rather thick, supravitaly stained, preparation which has been allowed to stand undisturbed for some minutes, the majority of the reticulated cells found are in the upper levels of the preparation. That this is not entirely due to the slow laking of the reticulated cells which is caused by the action of the stain can easily be demonstrated by allowing a test tube of oxydated anemic rabbit's blood to stand for a few hours until the cells settle out of the plasma and comparative equilibrium is reached. Then make preparations from the upper layers of the column of cells and from the bottom of the tube and ascertain the percentage of reticulated cells in each. A count will often show from 3 to 4 per cent of reticulated cells in a preparation made from the bottom of the column of blood, and from 40 to 50 per cent in the preparation made from the upper layers of the column of cells. The actual percentages vary, of course, with the percentage of reticulated cells in the blood and with the time the preparation is allowed to stand. The white blood cells and blood platelets are also concentrated in the upper layers of the column of cells. The process can be accomplished more quickly by centrifuging.

The reticulated cells are slightly more resistant to crenation than are the normal erythrocytes. In a supravitaly stained preparation which is allowed to crenate slowly, it is noted that the reticulated cells

31 Biffi, H. *Bull de Soc Med Ann* 79 8, 1908
(Quoted from Hawes)

are the last to be affected by the process. That this is not entirely due to the staining of these cells is indicated by the observation of unstained blood while crenation is in progress. The large pale cells are the last to be affected.

Much has been written on the resistance of the erythrocytes in anemic blood to various hemolytic agents. A brief review of the literature on this phase of the subject is given by Pepper and Peet,³² in connection with their studies on the resistance of reticulated erythrocytes to hypotonic sodium chlorid solutions. They concluded that there was no marked or constant difference in resistance to hypotonic salt solution between the blood of anemic rabbits and the blood of normal rabbits. I have not studied the resistance of anemic blood as compared to that of normal blood, but have merely studied the resistance of reticulated cells to hemoglobinolysis as compared to that of the nonreticulated cells in the same blood in hypotonic sodium chlorid solutions. The method used was to mix one part of oxylated blood with ten parts of salt solution, and then, at intervals of from two to twenty-four hours, stain specimens of blood from each mixture by withdrawing a drop in a pipet, placing it on a slide on which a drop of brilliant cresyl blue solution had been allowed to dry, covering and examining immediately. Salt solutions of from 0.3 to 0.5 per cent, varying by 0.01 per cent, and of from 0.5 to 1 per cent, varying by 0.05 per cent, were used.

It is necessary to make at least two preparations from each mixture because the unlaked cells settle to the bottom of the tube and the laked cells float in the solution above the column of unlaked cells. This fact may explain the diversity of results obtained by different investigators. The shadows of the nonreticulated cells can be seen in the preparations, and the laking of the reticulated cells can be recognized by the disappearance of the hemoglobin and by the behavior of the reticulum. When the reticulated cell is laked by distilled water and then stained with brilliant cresyl blue supravitaly, the reticular substance stains purple in the characteristic manner, except that no metachromatic granules are seen. It is, however, not in the form of a reticulum but is present in the cell as numerous small granules and fragments and usually a single rather large purple globule. The granules usually collect at the periphery of the cell and the globule may pass out of the cell and remain adherent to the membrane. The granules or fragments are irregular in size and shape and may move rapidly about in the laked cell. They stain in the color characteristic of the reticulum with all of the stains mentioned above as staining reticulum in the supravital

32 Pepper O. H. P., and Peet, M. M. Resistance of Reticulated Red Blood Cells. *Arch Int Med* 12:81 (July) 1913.

preparations The total amount of basophilic substance in the laked cell, however, appears to be much less than would result if the heavy mosslike wreath were fragmented in the cell If laked anemic rabbit's blood be examined unstained with a slightly lowered condenser the shadows of the laked cells can be seen and the fragments of reticulum are quite refractile and easily seen Their morphology does not differ from that seen in the stained laked reticular cells except that the large globule is not seen

This fragmented reticulum is seen in reticular cells laked by hypotonic salt solution regardless of the strength of the solution, provided the cell is laked In reticulated cells which are suspended in hypotonic salt solution of from 0.5 to 0.6 per cent sodium chlorid solution and then stained with brilliant cresyl blue as above described the reticulum is present as a smaller more compact wreath than that in reticulated cells in an isotonic medium In lower concentrations of salt solution the reticulum of the unlaked cells may be gathered together in the form of a dense ball or disc in the center of the cell The cells are swollen but contain hemoglobin In the laked cells, the picture is abruptly changed to the fragmented form of reticulum and the cells contain no visible hemoglobin

In the blood of rabbits rendered anemic by bleeding or by injection of phenylhydrazin hydrochlorid, hemolysis began at about 0.42 per cent and was almost complete at 0.3 per cent sodium chlorid solution In a drop of the solution taken from just above the column of cells in the mixture of oxyalted blood and 0.42 per cent salt solution and stained with brilliant cresyl blue as described above, laked reticulated cells and shadows of laked nonreticulated cells were seen In a drop taken from the bottom of the tube containing oxyalted blood and 0.3 per cent salt solution, a considerable number of unlaked hemoglobin containing cells were present When stained, a moderate number of these contained reticulum which was collected in the center of the cell as above described No cells were seen which resisted the action of distilled water In some earlier experiments, I examined only the cells in the bottoms of the tubes and drew the erroneous conclusion that the reticulated cells are more resistant to hemolysis than are the nonreticulated cells By examining both the cells which settled to the bottom and those which float free in the solution, no constant difference in resistance between reticulated and nonreticulated cells was noted This was true for the solutions of intermediate strengths as well as for those at the beginning and completion of hemolysis

SOME PROPERTIES OF THE BASOPHILIC SUBSTANCE

As noted above, if anemic rabbit's blood be laked with distilled water, the shadows of the young erythrocytes are seen to contain

refractile granules and strands which stain in the manner characteristic of the reticulum, except that no metachromatic substance can be seen. If the blood be allowed to remain in the distilled water and is examined from twenty-four to forty-eight hours later, the refractile granules and strands are not dissolved but are practically unchanged and can be seen unstained or stained in the usual manner. The mitochondria, on the other hand, disappear from the white blood cells, and after from one-half to one hour in distilled water cannot be stained with Janus green or seen unstained. In blood laked in various strengths of hypotonic salt solution and examined twenty-four hours later, the reticulum in the laked and unlaked cells is practically the same as that present in the laked and unlaked cells of a similar mixture examined immediately after laking.

If the blood be laked and stained simultaneously by mixing it with a solution of brilliant cresyl blue, 1:300 in distilled water all transitions between the typical mosslike wreath and the fragmented reticulum seen in cells which are first laked and then stained are seen in the same preparations. The transition stages apparently being the result of two opposing forces: (1) the stain, which tends to fix the reticulum in the form of a net, and (2) the water entering the cell, which tends to break it up into fragments. If the reticulum be stained first and then the cells laked by adding distilled water, the reticulum is not broken up as the cell is laked but retains its original morphology as a wreath or irregular net as though it had been fixed and rendered rigid by the stain. In supravitaly stained preparations which are ringed with petrolatum and allowed to stand for some hours, the reticulated cells are laked by the action of the stain, but the morphology of the reticulum remains unchanged. In forty-eight hours all of the cells are laked but the reticulum retains its original form and remains intact for days or as long as the preparation lasts.

If the blood cells be crenated in hypertonic sodium chlorid solution and then stained with brilliant cresyl blue or azur, the reticulum is stained in the normal form of a mosslike wreath, except that the outline of the wreath is distorted by the irregularities of the cell. If the blood be mixed with ten parts of 10 per cent sodium chlorid solution and allowed to stand twenty-four hours, and is then stained, the stain enters the crenated cells with difficulty and good preparations are not obtained. However, if from four to five parts of 1 per cent salt solution are added to the mixture of blood and 10 per cent salt solution, the crenated cells are quickly laked and the fragmented reticular substance can be seen unstained or stained in the usual manner and does not differ markedly from that of fresh laked erythrocytes.

This laking of crenated cells by solutions which are hypertonic to normal blood is apparently due to the fact that an osmotic equilibrium is established between the interior of the crenated cells and the surrounding medium and a reduction of the osmotic pressure in the surrounding medium causes first swelling and then laking of the cells. The threshold of laking being determined by the osmotic pressure within the cell. To explain this behavior, it is not necessary to assume that there has been any change in the permeability of the membrane of the erythrocyte. On the other hand, if the erythrocytes are first laked in distilled water or hypotonic salt solution and then mixed with hypertonic salt solution, it is noted that some of the laked shadows are markedly crenated while others are simply reduced in size but preserve their smooth contour. If the laked cells be left in the hypertonic medium, the crenated cells gradually round out and assume their normal contour. If the blood be laked only partially, so that many of the cells contain hemoglobin, and then mixed with a moderately hypertonic salt solution and examined some hours later, it will be seen that the hemoglobin containing cells remain crenated while the shadows of the laked cells are smooth and rounded though decreased in size. From the failure of some laked cells to crenate in hypertonic solutions, it is probable that the permeability of the cell membrane has been increased by the laking. The later rounding out of the membrane of the crenated laked cells when equilibrium has been established suggests that the cell has lost some of its rigidity and become more pliable.

In studying the resistance of the reticular substance to heat, oxylated blood was placed in a thermostat at a temperature of from 50 to 55 C and examined at intervals of from one-half to twelve hours. Stained with azur II, after one-half hour at 50 C the reticular cells were nearly all laked and the reticular substance did not stain in the characteristic netlike form but appeared as small isolated granules resembling the picture seen in punctate basophilia. The metachromatic granules could not be identified. In one hour, the reticulum had assumed the form characteristic of laked cells. After twelve hours at 50 C the reticular substance could still be stained in the laked cells. The mitochondria in the white blood cells disappeared in one-half hour at 50 C.

As demonstrated above, the reticular substance is not soluble in water or sodium chlorid solutions. That it is not soluble in the blood plasma is indicated by the fact that it can be demonstrated in oxylated or defibrinated blood which has been shed for days (sixteen days if kept on ice). In oxylated blood which has been shed for forty-eight hours or longer, the cells are gradually laked and the reticulum is stained in fragments in the manner characteristic for laked cells. As the

degeneration progresses, the fragments are smaller and less numerous and the cells also stain diffusely with the dye. If either dried or wet smears are immersed and fixed in ethyl alcohol, methyl alcohol, ether or chloroform, and stained with brilliant cresyl blue a punctate basophilia or basophilic fragments, or polychromatophilia is seen in a percentage of the erythrocytes corresponding roughly to the percentage of reticulated cells in the blood used. This indicates that the reticular substance is not soluble in ethyl or methyl alcohol, ether or chloroform.

If a drop of blood be mixed with a drop of 5 per cent acetic acid and examined with the condenser slightly lowered, the shadows of the hemolyzed red cells can be seen, and in some of these shadows, the percentage being about the same as the percentage of reticulated cells present in the blood, small refractile granules and broken strands can be seen. The degree of refraction of these fragments is less than that of the nuclei of the white blood cells, and is about the same as that of the protoplasm of the lymphocytes in the same preparation. These granules and fragments stain readily with crystal violet, methylene blue, malachite green, etc., which act in an acid medium, and are undoubtedly reticular substance in a fragmented form. The same reaction takes place in any concentration of acetic acid varying from 1 per cent solution to glacial acetic acid. If the preparation be ringed with petrolatum and examined twenty-four hours later, the refractile basophilic substance is seen to be unchanged.

With 5 per cent oxalic acid the red blood cells are hemolyzed and the fragmented reticulum can be seen in the shadows of the cells containing it, though it is slightly less refractile and more difficult to see than in cells treated with acetic acid. With a saturated aqueous solution of picric acid, the reticulum is fragmented and refractile and rendered visible. It is of interest to note that Erb³³ in 1865 treated fetal blood with acetic and picric acids and noted refractile granules in certain of the erythrocytes. He believed that the granules were remains of nuclei and regarded the cells containing them as transition stages between the red and the white blood cells. Erb undoubtedly observed the reticular substance many years before it was described and studied as basophilic substance.

As is illustrated in Schafer's textbook on microscopic anatomy,³⁴ if blood be mixed with an aqueous solution of tannic acid, the hemoglobin is slowly discharged from the cell and remains adherent to the cell membrane in the form of one or more irregularly spherical masses. If anemic rabbit's blood be mixed with the tannic acid solution, the

³³ Erb, W. Zur Entwicklungsgeschichte der roten Blut Körperchen. Virchow's Arch f path Anat **34** 138, 1865

³⁴ p 369

reticulated cells can be identified as the reticulum is rendered slightly refractile by the tannic acid and remains in the cell after the hemoglobin is discharged. It is not in the form of a network but is seen as slightly refractile granules throughout the cell. The shadows of the nonreticulated cells are clear.

As is noted by Fisher in the passage quoted from Meves, many acids, such as dilute hydrochloric or nitric, cause a precipitation¹¹ of the hemoglobin and the erythrocytes appear as though filled with refractile protoplasmic granules. The same acids in higher concentrations distort the erythrocytes and render them highly refractile. In either case, it is not possible to definitely identify the reticular substance unstained, and I was not able to stain it differentially in the cells. However, if the blood is laked in an excess of distilled water and centrifuged, and the sediment collected and washed in from three to four changes of water until the pink color has almost entirely disappeared, the hemoglobin is removed from the cells and no precipitate occurs on treating the washed laked cells with acids. The fragmented reticulum remains in the cell containing it and can be seen unstained or stained in the usual manner. If these cells be mixed with 5 per cent sulphuric acid, this fragmented reticulum is rendered highly refractile. The cell membranes of the reticulated cells also appear more refractile than the membranes of the nonreticulated cells. This may be due to minute particles of reticular substance adhering to the membrane. If the preparation is ringed with petrolatum and examined several hours later it is found to be practically unchanged.

If the washed-out cells be treated with 2 per cent nitric acid or 5 per cent hydrochloric acid, the picture is similar, although the reticular substance is less refractile than with 5 per cent sulphuric acid. With strong nitric acid and strong hydrochloric acid the laked cells are reduced in size, and their edges are irregular, but the fragments of reticular substance are not dissolved and can be seen in the shrunken cell shadows. In like manner, the reticular substance was found not to be soluble in 1 per cent chromic acid, 1 per cent phosphotungstic acid, 1 per cent phosphomolybdic acid, 1 per cent potassium permanganate, or Lugol's solution. Not only was it not dissolved, but its morphology was practically unchanged from that observed in the washed cells before subjecting them to the action of the various reagents, except that with the strong acids the particles are smaller in the contracted cells.

If the blood be mixed with 1 per cent sodium hydroxid solution, the erythrocytes are quickly dissolved and disappear entirely. The reticular substance is apparently dissolved also as no isolated reticular substance can be stained in the mixture. Schilling-Torgau¹⁹ observed

that reticulated erythrocytes treated with very dilute alkalis (1 drop of 1 per cent potassium hydroxid in from 30 to 50 drops 0.85 per cent sodium chlorid solution) and then stained supravitaly resulted in pictures resembling punctate basophilia and he interpreted his results as transition stages between reticulation and polychromatophilia. I was able to obtain the same pictures by treating the blood cells with dilute sodium hydroxid and then staining with brilliant cresyl blue. The reticular substance appears in the form of fine granules scattered throughout the cells and resembles the pictures obtained by heating the cells to 50 C for twenty minutes and then staining them supravitaly.

Millon's reagent was applied to anemic blood in the form of fresh blood, oxyalted blood, defibrinated blood, wet smears, dried smears and fixed smears. In a preparation made by quickly mixing a drop of defibrinated blood with a drop of Millon's reagent on a slide so that many isolated cells were present, then covering with a cover slip and heating slightly, the film of blood and reagent turns a light pink color. Certain of the erythrocytes contain refractile granules which are probably reticular substance. These granules are often seen to be colored a faint yellowish pink, but the reaction is very indefinite at best and no conclusions can be drawn from the test.

The reticular substance was tested for iron by the Prussian blue reaction and by McCallum's³⁵ ammonium hydrogen sulphid reaction. In no case was a positive Prussian blue obtained in any erythrocyte, although the nuclei of the white blood cells in the preparations gave a very definitely positive reaction. In using McCallum's ammonium hydrogen sulphid test for iron, fresh blood was mixed with glycerin one part and distilled water one part on a slide and a drop of freshly prepared ammonium hydrogen sulphid solution added. The preparation was covered with a cover glass and placed in a thermostat at from 48 to 50 C. In preparations examined four or five days later the deep green ferrous sulphid was seen to be present in the nuclei of the white blood cells. The cytoplasm of the white blood cells was, as a rule, clear but in a few cases a few green granules were present in the cytoplasm of large mononuclear cells. In no case were any green granules or diffuse green discoloration seen in any erythrocyte, although the preparations were kept in the thermostat as long as four weeks. The reticular substance persisted indefinitely in the erythrocytes containing it and could be seen as clear refractile granules and masses in the cells at the end of four weeks. As was noted by McCallum, the iron of the hemoglobin is so firmly bound that it is not liberated by either method and consequently does not affect the reactions.

³⁵ McCallum, A. B. Iron Compounds in Animal and Vegetable Cells. *Quart J Microscop Sc* 38 175, 1895.

According to Bensley³⁶ the reaction used for the microchemical detection of phosphorus in cells is not due to actual phosphorus in the cell but to the absorption of some molybdic acid from the nitric acid reagent. This molybdic acid forms compounds with the albumins in the tissues and gives the green blue reaction. Inorganic phosphorus is first affected then lecithin and then organic phosphorus. Tissues treated by the reagent (solution of ammonium molybdate in nitric acid) do not contain sufficient ammonium phosphomolybdate to give a blue or green color when treated with 0.25 per cent phenylhydrazine hydrochlorid solution. However, certain cells have been found to give a positive reaction, and so the reticulated cells were tested by the "phosphorus reaction". In no case was a positive reaction given by the reticular substance. In certain cases, when the action was prolonged, the erythrocytes were colored a pale bluish green and this is thought to be due to the lecithin in the cell membrane.

The potassium reaction of McCallum³⁷ was applied to fresh and dried smears of whole blood and washed blood corpuscles. The reagent³⁸ contains the hexanitrite of sodium and cobalt which precipitates potassium as a yellow triple salt. The preparation is washed in ice water from ten to twenty minutes and then treated with ammonium sulphid solution which forms a black precipitate of cobalt sulphid. The preparation is mounted in equal parts of water and glycerin. In a smear of anemic rabbit's blood treated by the above method, the erythrocytes appear as faint black rings and there are a variable number of small deep black granules arranged around the periphery of the rings. In certain of the erythrocytes, the granules are more numerous, larger and scattered diffusely through the interior of the ring. If the test be applied to whole blood in fluid medium, the shadows of the erythrocytes are seen and the granules are usually scattered irregularly over the inner surface of the membranes. In certain of the erythrocytes the granules are larger, and in the interior of the cell and often appear as though strung together in a loose irregular net. It was not found possible to stain a reticulum in these cells nor was it possible to demonstrate it in preparations which were first supravitaly stained and then treated with the reagent. However, the cells containing the increased amount of potassium were quite rare in normal rabbit's blood and were roughly proportional to the percentage of reticulated cells in the anemic

36 Bensley, R. R. Examination of the Methods for the Detection of Phosphorus Compounds other than Phosphates by Microscopical Methods, *Biol. Bull.* **10** 49, 1905.

37 McCallum, A. B. Distribution of Potassium in Animal and Vegetable Cells. *J. Physiol.* **32** 95, 1905.

38 Cobalt nitrite 20 gm., sodium nitrite 35 gm., water 75 c.c., dissolve, add 10 c.c. glacial acetic acid and dilute with water to 100 c.c.

rabbit's blood The presence of the small granules in all cells is explained by the fact that the erythrocytes of rabbits are quite rich in potassium It is reasonable to believe that the cells giving the stronger potassium reaction represent reticulated cells and that the reticular substance is rich in potassium

RELATION BETWEEN POLYCHROMATOPHILIA, PUNCTATE BASOPHILIA AND RETICULATION

Hawes, on a basis of comparative counts concluded that polychromatophilia, stippling and reticulation are all different manifestations of the same process Schilling-Torgau¹⁹ concluded that polychromatophilia and reticulation are different aspects of the basophilic substance and that punctate basophilia is a pathologic modification of this substance By fixing blood in an alcohol-ether mixture, or by treating reticulated cells with a heavy dilute potassium hydroxid solution in physiologic sodium chlorid solution and then staining, Schilling-Torgau was able to produce transition stages between reticulation and punctate basophilia These results of Hawes and of Schilling-Torgau were confirmed without difficulty As noted above, similar pictures of fragmented or granular reticulum were obtained by subjecting the cells to heat or to dilute acids and then staining supravitaly with brilliant cresyl blue, azur, etc However, it is to be noted that all of these pictures can be interpreted as a fragmentation of the reticulum due to the chemical or physical agent used, as the basophilic particles stain deeply and there is no diffuse staining of the erythrocytes containing them Hence, there is a definite gap between these pictures of fragmented reticulum and true polychromatophilia

If a smear of anemic blood be dried in air and then stained with azur, brilliant cresyl blue, Unna's alkaline methylene blue or any of the stains which stain reticulum supravitaly, it will be seen that the reticular substance does not stain as a typical mosslike wreath but as basophilic fragments and granules which are present throughout the cell and there is no diffuse staining of the ground substance of the erythrocyte If the smear is thoroughly dried by heat or by leaving for some hours or days before staining, the basophilic granules and particles are smaller If the smear is stained very soon after it is dried, the basophilic particles form irregular nets in the erythrocytes containing them If the smear is only partly dried and then inverted over a drop of the stain and examined as a supravital preparation, all transitions between the fragmented reticulum, the irregular nets and the typical mosslike wreath of reticulum can be seen in the same preparation The typical wreath forms are found in the cells which have not dried and do not adhere to the cover glass It seems quite definite, then,

that all are different aspects of the same substance and that drying the cells before staining interferes with the formation of the reticular net

In true polychromatophilia, the staining is diffuse and no granules or fragments are seen. In Wright's and other methods which give pictures of polychromatophilia, the cells are fixed by alcohol in the stain or fixed before staining. If Wright's stain be allowed to dry on a slide and then a drop of salt solution and blood be added and the preparation sealed with petrolatum and examined as a supravital preparation, no polychromatophilia is seen but a definite basophilic reticulum slowly appears in the basophilic erythrocytes. If freshly dried smears of anemic rabbit's blood be fixed in methyl alcohol, formalin vapor or osmic acid vapor, and then stained with Wright's stain or any of the stains which stain reticulum supravitaly it is found that not a particle of reticulum is seen in any of the erythrocytes but that a definite polychromatophilia is present and the percentage of the erythrocytes staining diffusely with the dye used is roughly proportional to the percentage of reticulated cells in the blood used.

If half of a dried smear be dipped in methyl alcohol and the smear be allowed to dry and then stained with azur or other reticular stain, it is seen that in the half which was dipped in alcohol the basophilic erythrocytes stain diffusely with the dye, and those in the half which was simply dried in air but not fixed contain fragments or an irregular network of reticulum. At the line of juncture between the fixed and the unfixed cells, transitions are seen, but the change is usually quite abrupt, a cell either being definitely polychromatophilic or exhibiting fragmented reticulum. By means of osmic vapor, however, a very definite and gradual transition can be traced between the two states. The method used was to fix a freshly dried smear by inverting it for two minutes over a small mouthed bottle containing 2 per cent osmic acid. The edges of the cover slip generously overlapped the mouth of the bottle, and, consequently, were exposed to very little of the osmic vapor, while the central portion of the smear was exposed directly to the osmic vapor. If a smear fixed in this manner is stained with azur II, it is found that the normal erythrocytes in the central portion of the smear are tinted with green. The basophilic erythrocytes in the central portion of the smear, which were exposed directly to the osmic vapor and consequently are well fixed, stain a diffuse purplish blue, and in them no basophilic particles are seen. In the cells at the extreme edges of the smear, which were scarcely influenced by the osmic vapor, the basophilic substance is present as fragments or as an irregular network and the ground substance does not stain diffusely. In the intermediate zone, where the cells were affected lightly by the osmic

vapor and only partly fixed, the basophilic erythrocytes stain diffusely purplish blue with the dye and also contain minute fragments or delicate nets of basophilic reticular substance, the polychromatophilia predominates toward the central portion of the smear and the fragmented reticulum predominates in the peripheral portions. Examination of such a preparation convinces one that polychromatophilia and the fragmented reticulum are different aspects of a common basophilic substance.

EXAMINATION OF FIXED CELLS

The fixation of small blocks of fresh tissue gave uniformly better results than did the fixation of fresh blood or of wet or dried smears. Consequently, only the results obtained by the study of stained sections of bone marrow of anemic rabbits and of livers of embryo cats will be considered. It was found that after fixation with fluids containing acids and staining for mitochondria with the acid fuchsin or iron hemotoxylin methods, the reticular substance of the young erythrocytes could be demonstrated in a fragmented form. This was true with the mitochondrial fixatives of Bensley and Meves, as well as with more general fixatives containing a higher percentage of acetic acid, such as Zenker's fluid. In such preparations, the young erythrocytes contain irregular, deeply stained, masses which resemble the fragments of reticular substance seen in young erythrocytes treated directly with acetic acid.

The osmic acid of Bensley's or Meves' fluids penetrates the bone marrow very poorly and it is only on the edges of the sections that the young can be positively differentiated from the mature erythrocytes because in the interior of even very thin blocks of tissue nearly all of the erythrocytes are partially laked and contain irregular deeply stained fragments which may be either reticular substance or hemoglobin or both. The fragments of reticular substance present in the young erythrocytes render it impossible to decide whether or not mitochondria are also present as they stain deeply with the mitochondrial stain.

In tissues fixed in a saturated solution of corrosive sublimate or picric acid in absolute alcohol containing 5 per cent acetic acid the erythrocytes were completely laked and the reticular substance could be stained in the membranes of the cells containing it by mitochondrial methods or by simply staining with methylene blue or any basic stain which stains reticular substance supravitaly. In such preparations, the reticular substance is seen as fine strands and small masses forming a delicate irregular network in the membrane of the laked erythrocyte. The interior of the mature erythrocytes is clear and unstained.

For the demonstration of mitochondria in young red blood cells the neutral formalin bichromate fixative of Regaud²⁴ gave by far the best results. It penetrates well and the mitochondria can be stained

by the iron hemotoxylin or any of the acid fuchsin methods. The danger of confusing the mitochondria with the fragmented reticulum is eliminated because, as was noted above, in young erythrocytes fixed in liquor formaldehyd the reticular substance is fixed diffusely in the cell and gives pictures of polychromatophilia.

The difficulty in studying red blood cells by means of the various mitochondrial technics for fixed tissues is that hemoglobin stains deeply with the stain which stains the mitochondria. This is equally true of acid fuchsin, iron hemotoxylin or Benda's violet stain. It was attempted to neutralize this by a preliminary deep staining of the hemoglobin by eosin, picric acid, organe G, or other contrasts tain. But, as all mitochondrial methods for fixed tissues with which I am familiar depend on staining the section deeply and then differentiating until the mitochondria stand out sharply, it was found that the preliminary counter staining of the hemoglobin either had little effect on the ultimate picture or caused the mitochondria to stain poorly.

However, the presence of the basophilic substance in the young red blood cells causes them to stain diffusely with the methyl green or toluidin blue of Bensley's²² acid fuchsin methods for mitochondria. As a result, they exhibit polychromatophilia, and the bright red mitochondria are easily seen in the bluish or greenish red background. Partially decolorizing the sections with picric acid before applying the counter stain renders the pictures somewhat clearer. It is consequently felt that the acid fuchsin staining methods of Bensley applied to tissue fixed by the neutral liquor formaldehyd method of Regaud are adequate for the demonstration of mitochondria in young red blood cells.

A careful study of quite a large series of well stained sections failed to reveal a single mitochondrial rod or granule in what could positively be identified as an erythrocyte. Occasional sections of hemoglobin containing cells were seen which contained a few mitochondria. It could not be determined positively that these were not slices of nucleated red cells which did not include the nucleus. The normoblasts with small pyknotic nuclei, as a rule, contained no mitochondria, although in some of them a few small mitochondrial rods and granules were present. In the younger red blood cells with larger normal appearing nuclei and little or no hemoglobin, mitochondria could constantly be demonstrated. In these young cells, the mitochondria are quite small when compared with the coarse mitochondria of the embryonic liver cells, but are larger than the minute mitochondria of the giant cells of the bone marrow. They vary in form from granules to curved rods of moderate length, the predominating forms being the rather short rods. They are not very numerous and are scattered irregularly through the cytoplasm, no constant relation to the nucleus or cell membrane being noted. As the

hemoglobin increases in the cells and the nuclei become pyknotic, the mitochondria decrease in size and number and gradually disappear entirely

DISCUSSION

That the presence of basophilic staining substance in erythrocytes is evidence of youth in the cells containing it is indicated by the facts that these cells are increased in states in which blood formation is stimulated by hemorrhage, etc., and decreased in states in which it is inhibited, not only in clinical states with decreased formation as in aplastic anemia, but also in experimental conditions as by repeated transfusions. The basophilic cells are present in increased numbers in embryonic blood and their percentage progressively decreases as the embryo approaches term. They are also present in large numbers in bone marrow and in embryonic livers where blood cell formation is in progress and the basophilic substance is demonstrable in nucleated red blood cells and can be seen to remain in the cell as the nucleus is being extruded.

In erythrocytes fixed by liquor formaldehyd, ethyl or methyl alcohol or osmic vapor and stained with basic dyes, the basophilic substance appears as polychromatophilia and is diffuse in the cell, no formed particles being visible with the oil immersion lens. In erythrocytes stained supravitaly with an appropriate basic dye, the basophilic substance is seen as a definite formed reticulum within the erythrocytes containing it and the cells do not stain diffusely with the dye used. In certain pathologic anemias a punctate basophilia is present. In this condition, after fixation of the blood cells by methyl alcohol, the basophilic substance appears as discrete granules in the cells containing it. My observations on punctate basophilia have been very limited, but they lead to the belief that the granules are formed of the same basophilic substance which in simple anemias gives pictures of polychromatophilia, and that because of the pathologic process this substance is aggregated into small granules in the cell.

In regard to the identity of polychromatophilia and reticulation in young erythrocytes the observation of Hawes¹⁸ that the percentage of each form in specimens of the same blood subjected to the appropriate technics is roughly the same was confirmed. The observations of Schilling-Torgau¹⁹ on the so-called transition forms produced by first treating the blood with dilute alkali solutions or fixing in alcohol ether mixtures and then staining were confirmed.

It is, however, to be noted that these forms can be interpreted as fragmented reticulum and that there is a distinct gap between them and true diffuse polychromatophilia. Similar fragmented forms of basophilic substance can be produced by subjecting the cells to heat,

various acids, or laking before staining supravitaly. It is also noted that by simply drying the cells and then staining with a basic stain the reticulum is demonstrated in a fragmented form. The degree of fragmentation is roughly proportional to the thoroughness with which the smears are dried before staining. In partially dried smears stained supravitaly all transitions between the fragmented forms and the true reticulum can be demonstrated in the same preparation. By varying the amount of fixation in different areas of the same smear with osmic acid or liquor formaldehyd vapor, it was found possible to demonstrate both fragmented reticulum and polychromatophilia in the same smear and in certain polychromatophilic cells a few fragments of reticulum could be demonstrated. Consequently, there is little doubt that the two are different aspects of the same substance.

The constancy with which the basophilic substance can be demonstrated under given conditions, and the fact that it can be seen in the laked cells without staining, prove that it is not a pure artefact due to the stain, but that it is a definite substance present in the cells containing it. However, the state in which this basophilic substance exists in the unaltered erythrocyte is unknown. It is obvious that either the diffuse polychromatophilia or the reticular net is due to the technic used. It has been noted above that with a stain which acts slowly (Janus green B) an irregular network is constantly observed, while with a stain which acts rapidly, such as brilliant cresyl blue or azur, in the great majority of reticulated cells of embryonic blood or of blood of rabbits rendered anemic by bleeding, the reticulum is present in the form of a mosslike wreath. It is further noted, that these two structures are different forms of the same substance. Thus, it appears possible for a supravital stain to determine the form in which the reticulum appears when stained. Also the reticulum when stained supravitaly is fixed in the form in which it originally appears, and tends to persist in that form even though it be stained a second time with a stain which, when applied to the unaltered red cell, tends to bring out a reticulum of entirely different morphology. Likewise, the stained reticular net retains its original form when the cell is laked in distilled water or even when the cell membrane has been destroyed and the reticular net floats free in the surrounding medium. The same is true of cells stained supravitaly which are dried on a slide or even fixed by alcohol, etc. The reticulum persists in the form in which it appeared in the supravital preparation.

On the other hand, in the unaltered erythrocyte the reticular substance has been shown to be very susceptible to reduction in osmotic pressure, being found collected in the central portion of the cell in hypotonic solutions which cause the cell to swell slightly. This suggests

that there is a certain amount of cohesion in the basophilic substance in the unaltered cell and that it is pushed inward by the water entering the cell from all sides. Then in the cells which are laked, the reticulum is found in definite fragments and masses which bear no resemblance to what one would expect to find if a preformed net had been broken up.

It is observed that the laking of an erythrocyte is not a gradual passing out of the cell of the hemoglobin but that there is a definite threshold for the cell concerned and that when this threshold is passed the cell is suddenly and violently laked and most but not all of the hemoglobin passes out of the cell. That some hemoglobin remains in laked cells can easily be demonstrated by treating them with dilute nitric acid when the hemoglobin is precipitated as large granules and easily seen in the shadows. It is believed that some of the reticular substance passes out of the cell with the hemoglobin, and that the remainder being insoluble in water collects in the refractile masses which can be seen without staining or stained with any of the dyes which stain reticulum.

In studying a fresh unstained preparation of anemic rabbit's blood an observer can pick out certain cells which, because of their size and color, seem to be young erythrocytes. These cells are slightly larger and slightly lighter in color than the surrounding cells. If the preparation is made with a very small drop of blood and pressed down so that the cells are actually flattened out it is possible to detect a slight difference in their structure from that of the normal erythrocytes. They are slightly more refractile. However, the difference is uniform throughout the cell and no formed masses of reticulum can be seen. Likewise, if the blood be examined in an isotonic medium with the ultramicroscope, it is not possible to see a reticulum in any of the erythrocytes. If an individual reticulated cell be watched carefully during the process of staining, the net can be seen to grow under the eye of the observer as though it were being formed by precipitation of substance from the surrounding medium.

In erythrocytes fixed by alcohol, liquor formaldehyd or osmic acid vapor the basophilic substance is distributed uniformly through the cell. These fixatives tend to fix intracellular products in situ, and it is difficult to conceive of their causing a definite network to break up into ultramicroscopic particles and become uniformly distributed through the cell. If they dissolved the network, its substance would pass out of the cell into the surrounding medium. It is consequently felt that sufficient evidence is at hand to warrant the conclusion that the reticular network as seen in supravitaly stained erythrocytes is formed during the process of staining, and that no such structure exists in the unaltered erythrocyte. The fragmented networks seen when smears are dried

and stained without fixing can be explained by the drying of the cells mechanically preventing formation of the typical mosslike wreath

The question next arises as to whether the diffuse basophilic substance is present as a thin layer on the inner side of the cell membrane or is distributed uniformly through the hemoglobin containing part of the erythrocyte. As noted above, in certain instances where the concentration of the surrounding medium has been raised very gradually by slow evaporation in ringed preparations the hemoglobin of some of the erythrocytes separates into two globules within the membrane. These globules may vary in size and resemble budding of the erythrocytes. In the bloods of two anemic patients who had undergone splenectomy some years before, this phenomenon was quite pronounced. What its significance is I do not know. However, when it occurs in supravitaly stained reticulated cells the reticulum is present in the globules of hemoglobin and not in the clear space between them and the cell membrane. Also, in sections of tissue fixed in liquor formaldehyd bichromate and stained to bring out polychromatophilia, it is not uncommon to find cross sections of erythrocytes. In these the polychromatophilia is uniform in the interior of the cell. It is consequently believed that the basophilic substance is uniformly distributed through the hemoglobin containing part of the erythrocyte and that, except in cases presenting punctate basophilia (stippling), its individual particles are so finely divided that they cannot be seen even with reflected light.

In regard to the chromatic granules of the reticulum, I am very uncertain as to their nature. So far as I am aware they have not been mentioned in American literature, but are quite well known to European hematologists. Schilling-Torgau believes that they arise in a definite region of the young red cell. Pappenheim states that they are soluble in water and consequently believes them to be lipoids. They stain red with brilliant cresyl blue or azur II, and hence stand out in sharp contrast to the purple or blue reticular net. However, they are rather difficult to stain in that one preparation may show them perfectly while the next half dozen preparations may show none at all. Azur II is the more reliable of the two stains for demonstrating them. In a good preparation, the chromatic granules appear as a variable number (from four to eight usually) of small spherical red granules scattered irregularly through the basophilic wreath and are present in the great majority of the reticulated cells.

The chromatic granules stain a little more slowly than the basophilic substance of the reticulum and then after from four to six hours slowly change color and cannot be differentiated positively from the rest of the reticulum as their morphology is not sufficient to identify them in the presence of the numerous small granules and masses of the net.

They apparently disappear from the cell, but careful watching leads to the belief that they change from red to purple and then in the azur II preparations to blue. In examining preparations stained with azur I, methylene blue, etc., it is felt that these same granules are present but are not stained differentially and cannot be identified positively.

In laked cells they cannot be stained by any method with which I am familiar, and in cells which are stained and then laked they cannot be identified. However, this does not necessarily mean that they are soluble in water and pass out of the cell. It is just as reasonable to suppose that the substance is present but stains the same color as the rest of the reticular substance since they can be observed to assume this color gradually, even under the best of conditions. If they are lipid granules, they should be quite highly refractile and easily seen in the unstained cell. But careful search with both transmitted and reflected light revealed no trace of them. Also fixation in osmic acid failed to bring them out. It is believed that their substance is diffuse in the unaltered cell and that the granules are formed under the influence of the stain used. They were observed in nucleated red blood cells stained supravitaly, but no evidence was seen which suggested that they arose in any special region of the cell. They were present in the reticulated erythrocytes of oxylated blood shed twenty-four hours previously and kept at room temperature. Hence they are not mitochondria.

It is well known that the basophilic substance gradually disappears from the erythrocyte as maturity is approached. In anemic rabbits which are permitted to recover their normal blood picture this process can be followed in the circulating blood but I was not able to determine definitely whether the reticular substance degenerated in the cell, entered into the formation of the cell membrane or passed out of the cell as basophilic material. Instances were seen where particles of reticulum were clinging to the outer sides of cell membranes and this suggests that it passes out of the cell in its original finely divided form and that the reticular fragments are formed on the outside of the cell during the process of staining.

On the other hand, the membrane of the mature erythrocyte is a very definite structure and is very different from the membrane of a young nucleated red cell. The membrane of the mature erythrocyte is thicker and less pliable. The membrane of the young nucleated cells appears to be hardly more than a limiting layer of the cell protoplasm. This membrane is formed as the cell matures, and it is possible that the basophilic substance enters into its formation. However, in the reticulated erythrocytes, where the reticular substance is most abundant, no difference can be made out microscopically between the membrane

and that of the mature erythrocytes. And it was shown that there is no constant difference in resistance to hypotonic salt solutions between the two types of cells. This indicates that the increased fragility of the erythrocytes seen in certain pathologic anemias is not due to the youthful cells in the blood but is due probably to some disturbance in the formation of the cell membrane. Another possibility is that there is a change in the plasma as it is shown that laking of erythrocytes is due to a relative change in environment rather than the exposure to any definite strength of salt solution. For instance, cells accustomed to a 2 per cent salt solution are laked in higher concentrations than are cells accustomed to a 1 per cent salt solution.

There is one very definite characteristic of the membrane of the young red cells which persists until after the reticular substance disappears from the cell. This is the stickiness of the outside of the membrane which causes reticulated cells to adhere to one another, to white cells, or to any object with which they are brought in contact, except mature erythrocytes. This tendency to agglutinate, or to adhere to other cells, offers a reasonable explanation of certain phenomena connected with the delivery of erythrocytes into the circulating blood. The young cells simply stick together where they are formed until the cell membrane is matured (apparently by a hardening of its surface) when the cell is delivered into the circulation. The theory of Bunting³⁹ and of Drinker⁴⁰ that the erythrocytes are forced into the circulation by the growth pressure of surrounding cells fails to account for the fact that, as a rule, only mature cells are delivered into the blood stream. This is explained by the fact that blood cells grow in nests with the mature cells on the periphery. However, a careful study of sections of active bone marrow leaves one unconvinced. A few nests are seen but nowhere is the arrangement so graded that pressure would deliver only the mature cells into the capillaries. The selection is apparently due to some inherent quality in the cell to be delivered.

It is believed that blood forms in a bony case because here the cells can stick together without being disturbed by external forces. Nowhere else could such a mass of loose cells and capillaries with so little supporting tissue exist. If pressure delivered the cells into the capillaries it is difficult to understand why the pressure, infinitesimal though it may be, does not simply collapse the very delicate capillaries where the blood is at very low pressure and in free communication with the blood stream.

39 Bunting, C. H. *The Regulation of the Red Blood Cell Supply*. Contribution to Medical and Biological Research, dedicated to Sir Wm. Osler, 2: 824. Paul Hoeber, New York, 1919.

40 Drinker, C. K. *Pathological Physiology of Blood Cell Formation and Blood Cell Destruction*. Oxford System of Medicine 2: 509. Oxford Press, New York, 1919.

outside the marrow I believe that Bunting and Drinker are correct in their opinion that the blood of the marrow circulates in closed spaces. If it were true that the blood of the marrow lies in open spaces, then the property of the young cells to stick together would be a perfect explanation for the delivery of the mature erythrocytes. The mature cells would simply be washed out by the slow current as soon as they lost their power of agglutination. However, with closed capillaries it is necessary to assume that the endothelium, which at one place or another is in direct contact with all types of cells in the marrow, exerts a selective action and opens temporarily to admit the mature erythrocytes with the firm nonadhesive membrane. Certainly not enough ameboid activity can be present in an erythrocyte to explain the process. It is here that all theories must of necessity stop until we have more facts on the subject. Possibly growth pressure is a factor in the actual delivery of the cells into the blood stream. However, the selective delivery of the mature cells must be due to some intrinsic quality in the cell to be delivered. The character of the membrane is probably the determining factor.

The stickiness of the membrane of young blood cells offers a reasonable explanation of the sudden disappearance of many of the young cells from the circulating blood after a crisis in which large numbers of young red cells suddenly appear and then disappear. The young cells simply adhere to the capillary walls in regions where the current is very slow. This theory has not yet been proved, but it is hoped that examination of the liver, spleen and bone marrow after a crisis will decide the matter.

The minute translucent flagellae observed by Kite⁴¹ on erythrocytes in Ringer's fluid can be seen in many cases in oxylated or fresh blood which is in a very slightly hypertonic medium. Kite apparently believed them to be living protoplasmic processes and observed erythrocytes which seemed to be actually swimming. These processes are best seen with reflected light in the dark field but it is possible to see them in transmitted light as they are slightly refractile. All transitions can be traced between the flagellae, small spicules and actual crenation. From the tendency of the fragmented reticular substance to adhere to the inside of the membrane in laked cells, it is believed that the inside of the membrane is covered with a semifluid layer. Under the influence of slightly increased osmotic pressure this substance may protrude in minute hairlike processes which, because of surface tension phenomena, may wave violently about in the surrounding

⁴¹ Kite, G. L. Some Structural Transformations of the Blood Cells of Vertebrates, *J. Infect. Dis.* **15** 319, 1914.

medium Their movements are not coordinated and in no sense resemble those of living flagellae They may retract or break off from the cell

It is believed that the hemoglobin part of the erythrocyte is in the state of a hydrophilic gel The elasticity of the cell, as a whole, which yields to pressure and immediately returns to its original shape when the pressure is released, conforms to this view Also the formation of vacuoles in the cell, the occasional formation of globules by the hemoglobin containing portion and the fact that in unlaked cells in which the reticular aggregates are being slowly formed under the influence of a supravital stain (Janus green B) the separate particles are stationary in the cell, and the behavior of the reticular substance under the influence of the hypotonic solutions which do not lade the cell, point to a gel rather than to a sol state When the cell is laked and water enters the cell, the gel is converted into a sol and particles of reticular substance move freely about in the cell and no vacuoles or globules are seen Unlaked cells which are crenated tend to remain crenated as long as they are subjected to the increased osmotic pressure but laked cells under the same conditions tend either not to crenate or to slowly resume their spherical contour It is believed that the physical state of the cellular contents as well as the increased permeability of the membrane of the laked cell has something to do with this behavior It was not found possible to demonstrate any formed structure in the mature erythrocytes It is believed that the basophilic substance of young erythrocytes is uniformly distributed through the hemoglobin containing gel The fact that erythrocytes can be cut across with a fine needle without permitting the escape of the contents can be explained by the fact that the semifluid sticky layer on the inner surface of the cell membrane would tend to seal the cut and cause the edges to stick together

Among the characteristics of chromatin noted by Halliburton⁴² and in Lee's *Microtomists Vade Mecum* are that it stains specifically with methyl green, dissolves in strong hydrochloric acid, and swells and loses its ability to stain when treated with strong sodium chlorid solution McCallum noted that chromatin always gives a positive iron reaction The reticular substance does not stain with methyl green, can be stained after remaining for twenty-four hours in 10 per cent sodium chlorid solution, and is resistant to strong hydrochlorid acid It does not give a positive reaction for iron, either by the Prussian blue or the ammonium sulphid method It is further distinguished from the nucleus by the fact that it is stained differentially from the

⁴² Halliburton, W D The Chemical Physiology of the Cell Brit M J. 1 501, 572, 627, 1893

nucleus by Janus green B, azur I, thionin, Unna's alkaline methylene blue, and toluidin blue. It is consequently believed that the reticular substance is of cytoplasmic origin.

The persistence of reticular substance in the cells in an unchanged form for days after the blood has been shed, its resistance to heat, to solution by water, acetic, nitric hydrochloric, oxalic and other acids, alcohol, ether and chloroform, its morphology, and its staining characteristics when treated with various basic dyes prove conclusively that it is not of mitochondrial nature, though it can be demonstrated in fragmented form by certain mitochondrial technic.

Pappenheim, on a basis of differential staining divides the cell into a nucleus, consisting of chromatin and parachromatin, and protoplasm, consisting of spongioplasm and paraplasm. The paraplasm is the true parenchyma of the cell and forms the secretion granules and specific cellular products such as hemoglobin. The spongioplasm is the inert cytoreticulum. On the basis of his differential staining methods he concludes that the basophilic (reticular) substance is of spongioplastic nature. His observations, as regards the staining characteristics of the reticulum were in the main confirmed by the author.

The fact that the reticular substance only appears in the cell after the hemoglobin has been formed also suggests that it is the less active part of the protoplasm and has nothing to do with the formation of the hemoglobin. Apparently, the hemoglobin as it is formed gradually replaces the active paraplasm permitting the inactive reticular substance to be separated out in such a manner that it can be precipitated in the form of a reticular net by the action of certain supravital stains. In the unaltered cell it is distributed through the homogenous gel in particles beyond the limits of microscopic vision.

As the hemoglobin accumulates the cell becomes senile. This is indicated by the increased density of the nucleus which becomes pyknotic before it is extruded. The increased potassium reaction of the reticulated cells suggests that the reticular substance is senile protoplasm since McCallum has noted that senile and degenerating cells give a strong potassium reaction. Mitochondria are characteristic of living protoplasm and disappear after the death of the cell. In young blood cells which contain little or no hemoglobin and a rather large pale nucleus, mitochondria can be demonstrated constantly. In normoblasts containing much hemoglobin and a small pyknotic nucleus only a few minute mitochondrial granules or none at all could be seen. In erythrocytes no mitochondria were seen. The mitochondria apparently disappear from red blood cells *pari passu* with the appearance of the hemoglobin.

I do not believe that mitochondria are transformed into hemoglobin. It is more reasonable to believe that granules common to practically every type of living cell have some general function common to all living protoplasm and that they do not enter directly into the formation of the specific product of the cell concerned. They probably play a part in the general metabolism of protoplasm. Their disappearance from the red blood cells is explained on the assumption that the hemoglobin actually replaces the protoplasm in the cell. It is objected that cells containing hemoglobin can be observed in various stages of mitosis. It is felt that here the replacement of the protoplasm has not been completed and that the division is in the nature of an agonal phenomenon. As a result of this division of a hemoglobin containing cell, the remaining protoplasm may become rejuvenated and form two daughter cells younger in type and containing proportionately less hemoglobin and more protoplasm and capable of further growth and proliferation. Or the tendency to differentiation and formation of hemoglobin may predominate and the nuclei become pyknotic, and are cast off, the cell membrane thickens, the protoplasm disappears, and the erythrocyte is formed. The need of the organism for erythrocytes probably has some influence on which of the above possibilities occurs in a given instance as well as the rapidity with which proliferation takes place.

The mature erythrocyte is the end stage of the process. It contains no nucleus and no evidence was seen which suggested that it contains any living protoplasm. The reticular substance of the young erythrocyte is believed to be the remnant of the protoplasm. Harrop's⁴³ observation that the normal mature human erythrocytes have no oxygen consumption measurable by present methods and that blood containing abnormal numbers of reticulated cells has an oxygen consumption proportional to the percentage of reticulated cells present is of interest here as it indicates that the erythrocyte contains living protoplasm after the nucleus is cast off. This is probably represented by the reticular substance which eventually disappears as the erythrocyte matures. The mature erythrocyte is apparently an envelope of complex structure containing hemoglobin distributed through a homogenous hydrophilic gel.

Clinically, the study of the mitochondria of the erythrocytes as an indicator of blood regeneration is not to be recommended because it has not yet been adequately demonstrated that they are ever present after the nucleus leaves the cell, though in rare instances it is possible that a few mitochondria may persist even in the erythrocyte.

⁴³ Harrop. Oxygen Consumption of Human Erythrocytes. Arch Int Med 23 745 (June) 1919

The study of the reticulated cells is recommended as a reliable indicator of the presence of young cells in the circulating blood. Since I have observed that the form of the reticulum varies in pathologic anemias and in anemias due to hemorrhage, it is felt that a careful study of the morphology of the reticulum in the various types of anemias may be of some value clinically.

Janus green B is not a specific stain for mitochondria in young erythrocytes because it stains the reticular substance in these cells. It is not so reliable a stain for reticular substance as brilliant cresyl blue or azur I or II. It is of value in certain cases in which the erythrocytes contain nuclear fragments, as in an hour or so these are stained pink and can then be differentiated from the green reticular substance present in the same cells.

Since this work was completed, an article by Cowdry⁴⁴ on the reticular material in developing blood cells has been published. It is to be noted that the reticular material studied by Cowdry is entirely different from the reticulum seen in supravitality stained erythrocytes. It has been described in many types of cells and is variously known as trophospongium, canalicular apparatus, reticular apparatus, etc., and, as Cowdry states, it probably represents areas of increased fluidity in the ground substance of living protoplasm. It is not seen in unstained or supravitality stained cells and is brought out as a reticulum by impregnation with osmic acid or silver nitrate, and as intracellular canaliculi by staining the more dense portions of the specially fixed cytoplasm of nearly all living cells. Cowdry was not able to demonstrate it in erythrocytes.

It is believed that Cowdry's failure to demonstrate this substance in erythrocytes is explained on the theory that mature erythrocytes have lost their cytoplasm and are not living cells and that young erythrocytes have lost all of their cytoplasm except the relatively inert basophilic substance and are dying cells. So far as I am aware this basophilic substance does not appear in any other cells in the body. But in no other cells is the nucleus lost and the protoplasm gradually replaced by a chemical substance (hemoglobin). The term reticular substance was used in this article because the basophilic substance is so generally referred to as reticulum. Basophilic substance is a much better term because it is shown that the reticulum of erythrocytes is formed during the process of staining and is due to the action of the stain on a basophilic substance distributed uniformly through the cell.

⁴⁴ Cowdry, E. V. The Reticular Material of Developing Blood Cells. *J. Exper. Med.* **33** 1, 1921.

CONCLUSIONS

1 Young erythrocytes differ from mature erythrocytes in that they have a lower specific gravity and tend to agglutinate or adhere to foreign subjects

2 The basophilic substance is characteristic of young erythrocytes. In the unaltered erythrocyte it is distributed uniformly through the hemoglobin containing part of the red blood cell, and when fixed and stained gives pictures of polychromatophilia

3 The reticulum is formed by the union of this basophilic substance with the supravital stain and its morphology varies with the stain used

4 The basophilic substance is not of nuclear origin. It is a protoplasmic constituent but is not of mitochondrial nature

5 Janus green B, used as a supravital stain, stains the basophilic substance of young erythrocytes in the form of an irregular net

6 The presence of mitochondria in young erythrocytes has not been satisfactorily demonstrated

7 Cytologically, the mature erythrocyte is not a living cell. The hemoglobin is contained in a hydrophilic gel which is surrounded by a definite membrane

UNCINARIAL NEPHRITIS~

F ROJAS, M D

AND

J TANCA MORENGO, M D

GUAYAQUIL, ECUADOR, S A

INTRODUCTION

It has long been recognized that anemia and dropsy are common symptoms of the more severe forms of uncinariasis, and edema is usually a prominent feature in fatal cases. Subcutaneous edema, ascites, hydrothorax, and hydropericardium are commonly found, and a general anasarca sometimes occurs. It has been impossible to establish the relation between the presence of hookworms in the intestines and the nephritis suggested by the above symptoms by means of the limited methods of investigation heretofore employed, but albumin, casts, leukocytes and injured epithelial cells have been found in the urine, and typical lesions of chronic parenchymatous nephritis have been found at necropsy in patients who have died in an edematous state.

The present study was undertaken to determine whether patients suffering from uncinariasis, and exhibiting edema, have any constant disturbance of renal excretory function, and whether this function is improved after anthelmintic medication. It had already been observed that, as a rule, the general health of the patient improves as soon as the parasites have been expelled, and that edema and anemia soon disappear. It is known that at least certain of the symptoms may be reproduced in animals by the injection of an emulsion of the heads of hookworms, or of the urine of individuals infested with the parasites. If any derangement of renal excretory function is present, it is probably due to toxic substances generated by the hookworms, and we should expect to find a normal or improved function after the parasites have left the body.

For the purposes of the present study, application has been made of the most recent knowledge concerning renal excretory function. The results of the study contribute support to the hypothesis that there is a form of nephritis due to hookworm infection, and that recovery from the infection is followed by recovery from nephritis.

*(From the Laboratories of the General Hospital and the University of Guayaquil)

The analysis of the following cases supports the findings of Ambard,¹ Widal and Weill concerning the chlorid threshold in nephritis with chlorid retention

METHOD OF STUDY

1 On the day of entering the hospital, samples of blood and urine of each patient were obtained in the usual manner to determine the Ambard's coefficient and the urea index. The sample of urine was also examined for albumin, casts, leukocytes and sugar. Blood viscosity and systolic and diastolic pressure were also noted. Ophthalmoscopic examination was made at the same time.

2 Salt poor diet (sodium chlorid, 2 gm daily), rest¹

3 When a balance between the chlorid intake (2 gm) and output was obtained, full doses of digitalin and theobromin were administered in the hope of lowering the chlorid threshold below the point reached on the salt poor diet with rest. At this time, a red cell count was made, and the Ambard's coefficient and urea index were again determined.

4 Sodium chlorid, 10 gm daily, was administered, and the chlorid excretion was followed. If the added chlorid was excreted with normal rhythm, 20 gm was administered daily and the excretion was followed. The weight of the patient was observed.

5 Anthelmintic treatment. The indication for discontinuing the treatment was the reduction of the hookworms in the fecal matter to a very small number.

6 Red cell count and the study of the urea coefficients on a salt poor balance.

7 The same as No. 4, plus the determination of the urea coefficients and an ophthalmoscopic examination.

Occasional doses of quinin were given during the time required for the study of each patient, as they had all been suffering from malarial fever before entering the hospital.

In the quantitative examination of the urea, the hypobromite method recommended by Ambard has been employed.

For plasma chlorids, an application of the Volhard principle made by Rappleye² was selected, this method has been effective in the hands of Van Slyke³

Blood tests for syphilis were made with the homohemolytic system of Noguchi⁴

1 During the stay of each patient urine was collected and examined in twenty-four hour periods in order to estimate the total excretion of sodium chlorid, to study the staircase rhythm and to compare the functional coefficients at the different rates of chlorid excretion.

2 Rappleye, W. C. *J Biol Chem* **35** 509, 1918.

3 Van Slyke, D. D., and Donleavy, J. J. *J Biol Chem* **37** 551, 1919.

4 Noguchi, H. *J A M A* **70** 1157 (April 20) 1918, *J Exper M* **28** 43, 1918.

The formulas used for calculating the urea coefficients were those given by Ambard and by McLean in their original contributions

For urea

$$\frac{Ur}{\sqrt{D \times \frac{70}{P} \times \sqrt{\frac{C}{25}}}} = K \qquad \frac{D \times \sqrt{C} \times 896}{Wt \times (Ur)^2} = I$$

Standard normal $K=0.07$

For chlorids

$$\frac{\text{Excess over the threshold}}{\sqrt{D \times \frac{70}{Wt} \times \sqrt{\frac{C}{14}}}} = K$$

Standard normal K (NaCl) = 0.11

Threshold = Plasma NaCl — Excess over the threshold

$$\text{Excess over the threshold} = K (0.11) \times \sqrt{D \times \frac{70}{Wt} \times \sqrt{\frac{C}{14}}}$$

(McLean)

$$\text{Threshold} = \text{Plasma NaCl} - \sqrt{\frac{D}{Wt} \times \frac{\sqrt{C}}{4.23}}$$

From a total of nine cases studied in the present work only two are taken for detailed discussion, although several of the others are summarized. As a matter of fact, the same disturbance of the kidneys was noted in every instance, and the slight variations observed were of minor importance and merely confirm the evidence of uncinarial nephritis and its disappearance as a result of the expulsion of the hookworms.

Special attention is demanded by the fact that a higher rate of chlorid excretion is obtained after a few days of treatment in anemic subjects who had previously exhibited a chlorid excretion insufficient to maintain a balance, without abnormal retention, even on the minimal intake of sodium chlorid necessary for a normal alimentation.

REPORT OF CASES

CASE 1 (9) — L. I., man, aged 24, was admitted to the hospital Feb 18, 1920, with a diagnosis of uncinariasis. He had lived twenty months in an uncinarial zone. During the last six months he complained of dizziness, vomiting of mucus in the morning, cramps in the legs, dyspnea on exertion, nocturnal pollakiuria, disorders of the vision, and ringing in the ears.

Examination — Noticeable pallor of the skin, edema of the face and feet, radial pulse 108, systolic murmur at base of heart, slight edema of optic papillae. The urine showed a trace of albumin and many finely and coarsely granular, cellular and hyaline casts, leukocytes, no sugar. Table 1 gives the urinary findings.

CASE 2 (8) — C. A. Z., man, aged 39, was admitted to the hospital Feb 18, 1920, with a diagnosis of uncinariasis. He had lived twenty-four years in an uncinarial zone. Anemia, dizziness and headache, cramps in the legs, nocturnal

pollakiuria, transitory edema of the eyelids and malleolus were the symptoms present some years ago. This man had previously entered the hospital complaining of uncinarial anasarca, but he had recovered under anthelmintic therapy. At the present time, marked anemia and slight edema of the feet and legs were noted, radial pulse 84, papillary edema and congestion in the ocular fundi. The urine showed the same findings as in Case 1.

Since Cases 1 and 2 are similar, they will be discussed together. From the investigations made by Ambard, Widal and Weill,⁵ it is known that the same general laws apply to the excretion of urea and of sodium chlorid, but with the important difference that there is a

TABLE 1—URINARY FINDINGS IN CASE 1*

Date	Urine			NaCl Intake, Gm	Blood Viscosity	Ambard's Coefficient	Urea Index	Urea per Liter of Plasma, Gm	Sodium Chlorid		Threshold	
	Quantity per 24 Hrs, Cc	NaCl per Liter, Gm	NaCl in 24 Hrs, Gm						Per Liter of Plasma, gm	Rate of Excretion per 24 Hrs, Gm	Ambard, Gm	McLean, Gm
Feb 18					2 0	0 059	184	0 271	6 45	13 64	6 12	6 05
Feb 19	2,080	5 00	10 40									
Feb 20	1,810	4 00	7 24									
Feb 21	2,000	3 00	6 00									
Feb 22	2,050	1 50	3 07									
Feb 23	2,450	1 00	2 45									
Feb 24	2,000	1 00	2 00									
Feb 25	1,340	1 60	2 14		2 2	0 069	134	0 242	6 41	4 39	6 22	6 20
Feb 26	750	5 00	3 75	10								
Feb 27	1,580	7 00	11 06	10								
Feb 28	2,240	3 00	6 72	20								
Feb 29	3,330	5 50	18 31	20								
Mar 1	3,070	7 00	21 49	20								
Mar 2	2,580	8 00	20 64	20								
Mar 3	3,300	8 00	26 40	20								
Mar 28	1,300	2 00	2 60		2 2	0 066	144	0 200	5 76	12 00	5 453	5 43
Mar 29	2,450	5 00	12 25	15								
Mar 30	4,700	8 00	37 60	35								
Mar 31	4,820	14 50	69 89	70		0 100	62	0 178	6 37	195 13	3 82	4 54
April 1	6,810	14 60	99 42	100†								
April 2	5,170	14 10	72 89	120								
April 3	1,120	3 50	3 92									

* Medication Feb 24, digitalin, 0 0006 gm, Feb 24, theobromin, 2 5 gm, from March 4 to 21, thymol, 12 gm

† April 1, fever, with temperature 40 3 C, it was not possible to complete intake

threshold for chlorid excretion. If the concentration in the plasma falls to a value below the threshold, the excretion of chlorid practically ceases, therefore, it is the sodium chlorid above the threshold which determines the rate of excretion, in accordance with those laws that hold in the case of urea. A greater excess over the threshold means a correspondingly greater rate of excretion.

5 Widal, F, and Javal, A. *Compt rend Soc de biol* **57** 301, 1904, Ambard, L, and Weill, A. *Semaine med* **32** 217, 1912, Widal, F, Ambard, L, and Weill, A. *Semaine méd* **32** 361, 1912, Ambard, L, and Chabanier, H. *Arch urol clin de Necker*, **1** 248, 1913

The excess over the threshold can be increased by the following changes By the lowering of the threshold, by the elevation of the actual plasma sodium chlorid, or by the fall of the threshold and the elevation of the actual plasma sodium chlorid at the same time In certain cases an increase in the excess above the threshold occurs in spite of a decrease in the actual plasma sodium chlorid as the result of an even greater fall of the threshold Indeed, the characteristic of normal kidneys is their capacity to move the threshold and actual plasma chlorid values in an effective manner to maintain in equilibrium the intake and output of chlorid without abnormal retention

TABLE 2—URINARY FINDINGS IN CASE 2*

Date	Urine			NaCl Intake, Gm	Blood Viscosity	Ambard's Coefficient	Urea Index	Urea per Liter of Plasma, Gm Gm	Sodium Chlorid		Threshold	
	Quantity per 24 Hrs, O c	NaCl per Liter, Gm	NaCl in 24 Hrs, Gm						Rate of Excretion per	Per Liter of Plasma, 24 Hrs, Gm	Ambard, Gm	McLean, Gm
Feb 18					2 5	0 082	93	0 305	6 55	16 32	6 00	6 07
Feb 19	2,100	5 50	11 55									
Feb 20	1,800	4 20	3 36									
Feb 21	1,300	0 50	0 65									
Feb 22	1,400	1 00	1 40									
Feb 23	910	1 75	1 59		2 5	0 093	73	0 237	6 18	2 19	6 03	6 07
Feb 24	1,560	1 50	2 34	10								
Feb 25	1,400	4 00	5 60	10								
Feb 26	1,750	5 00	8 75	10								
Feb 27	1,900	7 00	13 30	10								
Feb 28	2,180	5 00	10 90	20								
Feb 29	2,190	8 00	17 52	20								
Mar 1	2,000	7 00	14 00	20								
Mar 2	1,860	11 00	20 46	20								
Mar 3	2,100	10 20	21 42	20								
Mar 15	2 060	4 00	8 24									
Mar 16	1,450	5 00	7 25									
Mar 17	1,000	2 00	2 00									
Mar 18	1,680	1 00	1 68		2 7	0 090	79	0 253	5 63	2 73	5 42	5 47
Mar 19	770	7 50	5 77	10								
Mar 20	1 060	10 20	10 20	10								
Mar 21	1,780	9 00	16 02	10								
Mar 22	1,900	11 50	21 85	20								
Mar 23	1,660	14 80	24 56	20	3 1	0 082	93	0 243	5 78	26 64	4 97	5 07

* Medication Feb 22, digitalin, 0 0006 gm , Feb 22, theobromin, 2 gm , from March 4 to 14, thymol, 9 gm

On the contrary, the renal incapacity to excrete chlorid would result from an abnormal and permanent elevation of the threshold and the impossibility of lowering it to within normal limits in order to increase the excess above the threshold and to induce a sufficient rate of excretion to balance chlorid ingestion For it is not possible for the actual plasma sodium chlorid to rise above certain invariable limits For instance, an individual in whom the greatest excess over the threshold is capable of generating only a rate of excretion of 10 gm sodium chlorid, if the ingestion be increased to 20 or 30 gm , will exhibit

a retention of 10 or 20 gm, since the threshold cannot be lowered further. Furthermore, the concentration in the blood and the rate of excretion of urea and of chlorids depends not only on the ingestion of nitrogen or chlorid, but also on the balance between the ingestion and elimination of fluids.

With this basis established, together with the graphic and tabular presentation of two of the nine cases studied in the present work, it is not necessary to enter into a longer discussion. A marked insufficiency of chlorid excretion is demonstrated in these patients, which in certain instances is associated with a disturbance of urea excretion.

In Case 1, when infected by hookworms, there is shown a normal rhythm of retention of sodium chlorid, but the failure of the kidneys when exhibiting a rate of excretion of 13 gm sodium chlorid, is shown by the high values of the threshold and of the actual plasma sodium chlorid. The same subject, after treatment, excreted at a similar rate, 12 gm sodium chlorid, stimulated only by a threshold and actual plasma sodium chlorid of 5 453 and 5 760. After treatment a rate of excretion of 195 gm was obtained by means of a rise to 6 370 gm of the actual plasma sodium chlorid and the remarkable fall of the threshold to an unusual level, 3 82 gm.

In Case 2, when infected by hookworms, there is demonstrated an abnormal rhythm of retention and high values of the threshold and actual plasma sodium chlorid which induce, however, only a rate of excretion of 12 gm sodium chlorid, while after the expulsion of the hookworms, a higher rate of excretion was obtained with a simultaneous fall in the values of the threshold and actual plasma sodium chlorid.

CASE 3 (7) —J A R, man, aged 23, was admitted to the hospital Feb 18, 1920, with a diagnosis of uncinariasis. He had lived five years in an uncinarial zone. Symptoms which had lasted several months were cramps in the legs, ringing in the ears, nausea and vomiting of a bitter and salty fluid in the morning, headache.

Examination —Revealed noticeable pallor of the skin and conjunctiva, edema of the face and feet, radial pulse 120, systolic murmurs at the apex of the heart, enlarged spleen, edema of the optic papillae. In the urine were the same findings as those described in previous cases.

February 18. Ambard's coefficient 0076, actual plasma sodium chlorid, 6 44 gm, threshold, 5 94 gm, rate of excretion, 12 14 gm.

February 23. Ambard's coefficient 0083, actual plasma sodium chlorid, 6 32 gm, threshold, 6 06 gm, sodium chlorid per twenty-four hours, 3 36 gm, red blood cells, per cmm, 1,500,000, blood pressure systolic 110, diastolic 45, blood viscosity 2 1.

Medication —February 22. Digitalin, 0 0004 gm, theobromin, 1 50 gm. From March 5 to 16, 9 gm thymol in three doses.

March 18. Ambard's coefficient, 0074, actual plasma sodium chlorid, 6 16 gm, threshold, 6 01 gm, sodium chlorid per twenty-four hours, 3 27 gm, blood pressure systolic, 106, diastolic, 68, blood viscosity 2 4.

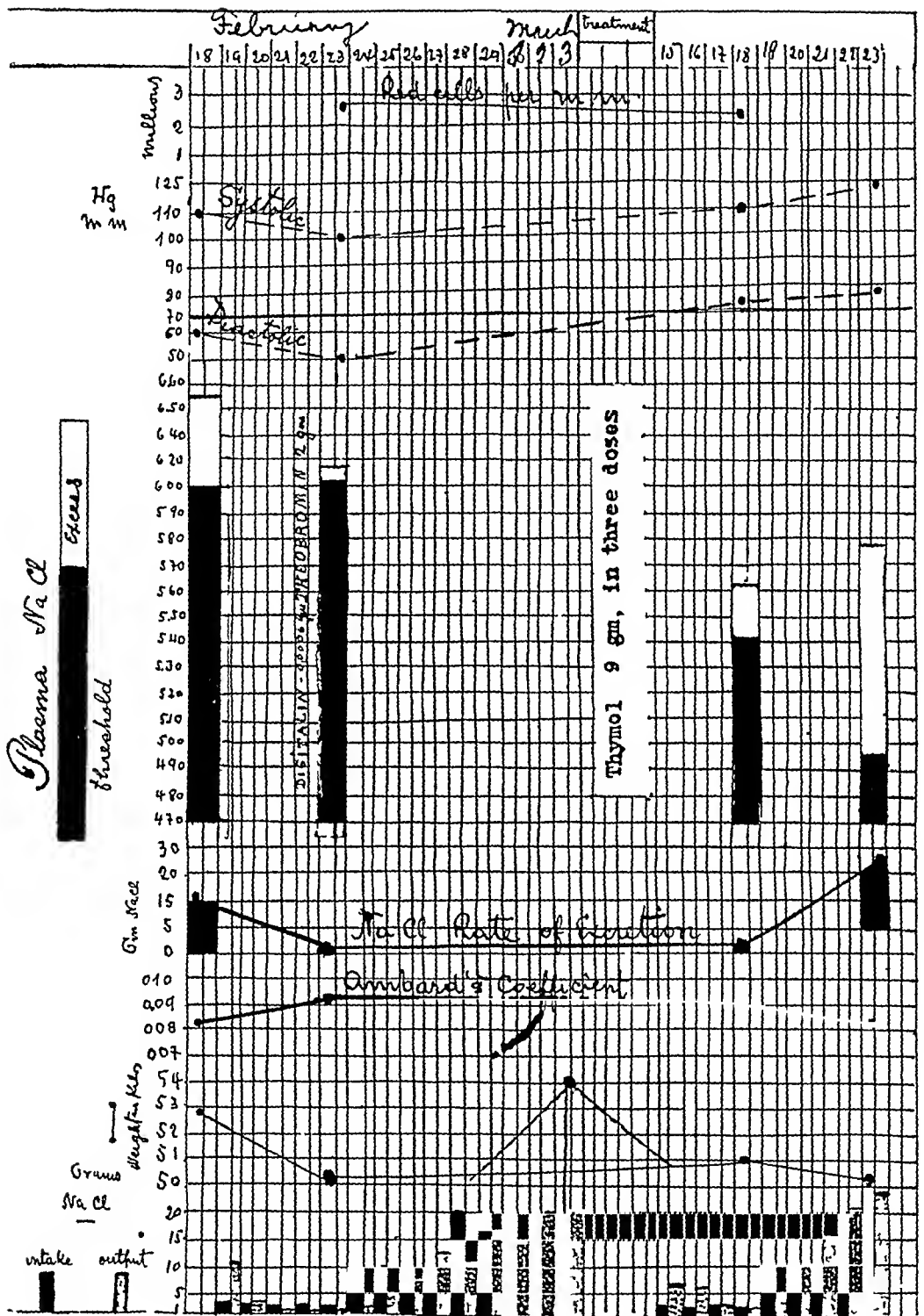


Fig 2—Graphic representation of data in Table 2

March 28 Ambard's coefficient, 0.075, actual plasma sodium chlorid, 6.01 gm, threshold 5.44 gm, rate of excretion, 17.95 gm, red blood cells per c mm, 1,500,000, blood pressure systolic, 104, diastolic, 58, abnormal rhythm of retention on 10 gm sodium chlorid before treatment, normal rhythm of retention on 10 and 20 gm sodium chlorid after treatment

CASE 4 (6)—S H, boy, farmer, aged 17, was admitted to the hospital February 16, with a diagnosis of uncinariasis. He had been resident three years in an uncinarial zone. Chilliness, headache, nausea and vomiting, dizziness, ringing in the ears, edema of the face and lower extremities were the symptoms.

Examination—Severe anemia, radial pulse, 108, systolic murmurs at base of heart, pollakiuria, enlarged spleen, trace of edema in optic papillae. In the urine were albumin and casts as described in previous cases.

February 16 (day of entering hospital) Ambard's coefficient, 0.066, actual plasma sodium chlorid, 6.55 gm, threshold, 5.94, sodium chlorid per twenty-four hours, 21.19 gm, blood pressure systolic, 115, diastolic, 55, blood viscosity, 1.9.

February 24 Ambard's coefficient, 0.066, actual plasma sodium chlorid, 6.35 gm, threshold, 5.98 gm, sodium chlorid per twenty-four hours, 9.97 gm, blood pressure systolic, 108, diastolic, 50, red blood cells per c mm, 1,020,000.

Medication—February 23 Digitalin, 0.0004 gm, theobromin, 1.50 gm, thymol, 9 gm in three doses.

March 24 Ambard's coefficient 0.070, actual plasma sodium chlorid, 5.66 gm, threshold, 5.24 gm, sodium chlorid per twenty-four hours, 8.91 gm, blood pressure systolic 96, diastolic 54, red blood cells per c mm, 1,490,000, blood viscosity, 3.5.

March 27 Ambard's coefficient, 0.061, actual plasma sodium chlorid, 6.01 gm, threshold, 5.31 gm, rate of excretion, 31.51 gm, blood pressure systolic, 100, diastolic, 76, sodium chlorid excretion obtained on salt poor diet with rest, 22.50 gm, normal rhythm of retention on 10 gm sodium chlorid, abnormal rhythm of retention on 20 gm sodium chlorid.

CASE 5 (5)—R Z, man, farmer, aged 25 years, was admitted to the hospital February 6. He had been resident twelve years in an uncinarial zone. He complained of dizziness, general weakness, ringing in the ears, and dyspnea on exertion during the last year, severe anemia, nausea and vomiting.

Examination—Systolic murmur at base of heart, radial pulse 108 and oliguria. In the urine were the same findings as in the foregoing cases. (The study of this case was incomplete owing to the departure of the patient.)

February 6 Ambard's coefficient, 0.140, actual plasma sodium chlorid, 6.40 gm, threshold, 5.95, sodium chlorid per twenty-four hours, 3.52 gm, blood pressure systolic, 110, diastolic, 60, red blood cells per c mm, 1,980,000, blood viscosity, 2.6.

Medication—Thymol, 9 gm in three doses.

March 3 Ambard's coefficient, 0.090, actual plasma sodium chlorid, 6.19 gm, threshold, 5.71 gm, sodium chlorid per twenty-four hours, 8.21 gm, blood pressure systolic, 108, diastolic, 66, blood viscosity, 3.1, red blood cells per c mm, 2,260,000. This patient showed an abnormal rhythm of retention on 10 gm sodium chlorid.

CASE 6 (4)—A V, man, farmer, aged 21, was admitted to the hospital January 29. Resident seven years in uncinarial zone. For one year previous he had complained of headache, ringing in the ears, anemia. Edema appeared in the last four months which increased to anasarca with marked anemia, chilliness, nausea and vomiting, dizziness, mentality was subnormal, he was insensitive to slight stimuli. Papillary edema of both optic nerves. In the urine were the same findings as previously described.

Salt poor diet and rest caused an excretion of 221.93 gm sodium chlorid and a loss of weight of 10.88 kg. After anthelmintic treatment he was able to excrete enormous amounts of salt without discomfort.

January 29 Ambard's coefficient, 0.130, actual plasma sodium chlorid, 6.20 gm, threshold, 5.55 gm, rate of excretion, 8.85 gm, blood pressure systolic 110, diastolic, 50, blood viscosity, 2.3

Medication—February 14 Digitalin, 0.0004 gm, theobromin 1.50 gm

February 15, digitalin, 0.0004 gm

February 15 Ambard's coefficient, 0.086, actual plasma sodium chlorid, 6.22 gm, threshold, 5.91 gm, rate of excretion, 4.89, blood pressure systolic, 100, diastolic, 55, blood viscosity, 2.5, red blood cells per cmm 1,400,000

Medication—Thymol, 9 gm in three doses

March 14 Ambard's coefficient, 0.083, actual plasma sodium chlorid, 6.10 gm, threshold, 5.22 gm, rate of excretion, 25.33 gm, blood pressure systolic, 96, diastolic, 56, blood viscosity, 3.1, red blood cells per cmm 1,480,000

March 20 Ambard's coefficient, 0.071 (25 gm of urea added to diet), actual plasma sodium chlorid, 6.02 gm, threshold, 5.26 gm, rate of excretion, 26.07 gm, blood pressure systolic, 104, diastolic, 52, blood viscosity, 4, red blood cells per cmm, 1,940,000

March 21 Ambard's coefficient, 0.081, actual plasma sodium chlorid, 6.12 gm, threshold, 4.79 gm, rate of excretion, 60.91 gm, blood pressure systolic, 104, diastolic, 52, blood viscosity, 3.9

Retention of 221.93 gm sodium chlorid Normal rhythm of retention on 10 gm sodium chlorid before treatment Abnormal rhythm of retention on 20 gm sodium chlorid before treatment After anthelmintic therapy it was possible to obtain a normal rhythm of retention even on 57 gm sodium chlorid

In the other three cases, the same results were obtained Case 7 (3) was a syphilitic man to whom mercuric cyanid was administered during the time required for observation, after which there was still a weakly positive Noguchi test in the blood No patient showed edema in the optic papillae after treatment

That thymol and sodium sulphate are not the direct cause of the fall in the values of plasma sodium chlorid and of the threshold is demonstrated in the following observations made in a normal subject

1 March 9, 1920, Ambard's coefficient, 0.066, actual plasma sodium chlorid, 5.89 gm, threshold, 5.50 gm, rate of excretion, 24.85 gm, sodium chlorid per liter of urine (concentration), 3.50 gm, blood pressure systolic, 118, diastolic, 64, blood viscosity, 4.8

Medication—March 10, 1920 Thymol, 4 gm, sodium sulphate, 120 gm

2 March 11, 1920 Ambard's coefficient, 0.060 (urea ingestion 25 gm), actual plasma sodium chlorid, 5.92 gm, threshold, 5.44 gm, rate of excretion, sodium chlorid, 25.49 gm, (concentration sodium chlorid, 12.10 gm), blood pressure systolic, 116, diastolic, 70, blood viscosity, 5.2

Threshold 2 is lower than Threshold 1 owing to the higher concentration of sodium chlorid in the urine, since the rate of excretion is approximately the same Rate of excretion Threshold 2 calculated with concentration Threshold 1 gives 0.38 as the excess, which is the same as the excess for Threshold 1, so calculated the Threshold for Threshold 2 would be 5.54 gm

CONCLUSIONS

In the nine subjects studied, hookworm infection caused nephritis with chlorid retention The index of urea excretion was low in three cases

The return to normal took place as soon as anthelmintic therapy expelled the hookworms, although the red cell count was still low

The etiology of this nephritis is the infection by hookworms, its pathogenesis remains unknown⁶

6 Additional bibliography not specifically mentioned in text Chabanier, H, and Lobo-Onell, C Arch urol clin de Necker, **1** 235, 1913, Legueu, F, Ambard, L, and Chabanier, H Arch urol clin de Necker, **1** 275, 1913, Peabody, F W J Exper M **17** 71, 1913, McLean, F C, and Selling, L J Biol Chem **19** 31, 1914, McLean, F C J Exper M **22** 212, 366, 1915, J Exper M **26** 181, 1917, J A M A **66** 415 (Feb 5) 1916, J A M A **69** 437 (Aug 11) 1917, Ambard, L Physiologie normale et pathologique des reins, Paris, 1914, Ed 2, 1920, Castaigne, J J med franc, **8** 441, 1919, Ashford, Bailey K, and Gutierrez Igaravidez, P Uncinariasis in Porto Rico, Washington, 1911, Gov't Printg Off, Senate Document, No 808

COMPARATIVE STUDIES IN THE CHEMISTRY OF BLOOD AND CEREBROSPINAL FLUID*

GRETE EGERER-SEHAM, PH D, AND C E NIXON, M D
MINNEAPOLIS,

Chemistry plays an ever-increasing rôle in the practice of medicine, in the field of metabolism it is the alpha and omega. Medical chemistry deals largely with studies of body fluids and gases. The urine, because of its accessibility, was the first to be subjected to investigation, while the cerebrospinal fluid, because of its inaccessibility, has received relatively little attention to date.

Although chemical changes undoubtedly do accompany infectious and protozoal diseases, the paucity of information concerning the nature of chemical changes in infections, and the complex nature of the proteins themselves, have barred, to a large extent, direct chemical approach to the study of infection. As a result, progress has come along other and indirect lines, namely, through the development of immunology.

Because of the frequent involvement of the central nervous system in syphilis, and because of the relatively simple chemical character of cerebrospinal fluid, it would appear probable that chemical studies of this fluid in syphilis would yield facts of diagnostic significance. To date the chemical studies resulting in information of clinical significance have been largely relating to the globulin content and to substances affecting the colloidal gold curve.

Since the cerebrospinal fluid ultimately is derived from blood, it is important to determine the mechanism of its production, that is, whether by filtration or active secretion. In any event, it is certain that marked changes in the content of the blood are reflected to a greater or lesser degree in the cerebrospinal fluid. On the other hand, it is apparent that diseases of the structures concerned in the formation and fate of the cerebrospinal fluid may bring about changes in its chemical constitution and a disturbance in normal chemical relationship of the blood and cerebrospinal fluid. It would seem not unlikely that various diseases with predilection for certain structures and regions of the central nervous system should show more or less characteristic disturbances in the normal relationship of these fluids.

* From the Department of Medicine, The Medical School, University of Minnesota.

* This investigation was carried on under a grant from the Interdepartmental Social Hygiene Board and under the direction of Dr. L. G. Rowntree.

Accordingly, quantitative studies were made, as far as possible in the same individuals, of the acid base equilibrium, of sugar, urea, creatinin content, and enzymatic activity of blood and cerebrospinal fluid

THE SUGAR CONTENT OF CEREBROSPINAL FLUID

Schloss and Schroeder,¹ in a review of the literature on sugar content in cerebrospinal fluid, state that "it is generally assumed that the reducing agent in cerebrospinal fluid is dextrose, the sugar of blood and body fluids. A review of the literature, however, discloses that there has been considerable divergence of opinion. Halliburton,² whose views are quoted in several current textbooks, concluded that the reducing substance was pyrocatechin or a pyrocatechin derivative. Connall³ states that it is galactose, although he gives no references or experimental data to uphold his view. The observations of Narwatzki,⁴ Zdarek,⁵ Panzer,⁶ Rossi,⁷ and others, however, indicate that the reducing agent is a fermentable dextrorotatory sugar, presumably dextrose." Observations made by Schloss and Schroeder themselves indicate that there is no basis for the belief that the reducing substance in normal cerebrospinal fluid is pyrocatechin. Moreover, their results indicate that this agent is a monosaccharide, probably dextrose.

QUANTITATIVE DETERMINATION OF SUGAR

Narwatzki,⁴ using Alladin's method, determined sugar in 121 c c of spinal fluid preserved with alcohol. His findings, as well as those of other investigators, are tabulated in Table 1.

A study was made of the sugar content of ninety-seven spinal fluids using the method of Lewis and Benedict⁸ as modified by Myers and Bailey.⁹ Two c c of spinal fluid were used, except in rare instances

1 Schloss and Schroeder. Nature and Quantitative Determination of the Reducing Substance in Normal and Pathologic Spinal Fluids, *Am J Obst* **77** 548, 1915, *Am J Dis Child* **11** 1 (Jan) 1916

2 Halliburton. Textbook of Chemical Physiology and Pathology, London, 1891

3 Connall. A Study of the Cerebrospinal Fluid in the Infective Diseases of the Meninges, with Special Reference to Cerebrospinal Fever, *Quart J M* **3** 152, 1909

4 Narwatzki. Zur Kenntniss der Cerebrospinalflüssigkeit, *Ztschr f Physiol Chem* **23** 533, 1897

5 Zdarek. Ein Beitrag zur Kenntniss der Cerebrospinalflüssigkeit, *Ztschr f physiol Chem* **35** 211, 1902

6 Panzer. Zur Kenntniss der Cerebrospinalflüssigkeit, *Wien klin Wchnschr* **14** 805, 1899

7 Rossi. Sulla natura della sostanza riducente contenuta nel liquido cefalorachidea, *Clin Med Ital* **38** 422, 1899

8 Lewis, R. C., and Benedict, S. R. A Method for the Estimation of Sugar in Small Quantities of Blood, *J Biol Chem* **20** 61, 1915

9 Myers, V. C., and Bailey, C. V. B. The Lewis and Benedict Method for the Estimation of Blood Sugar, with Some Observations Obtained in Disease, *J Biol Chem* **24** 147, 1916

when only 1 c c was available. The determinations were carried out within half an hour after the puncture. The spinal fluid was collected in test tubes of 5 c c capacity, corked with rubber stoppers, and kept in the ice box until analyzed.

Of the ninety-seven fluids of our series obtained from patients on whom lumbar puncture was done as a diagnostic procedure, nine might be considered normal, there being present no organic disease which would be likely to modify the cerebrospinal fluid. (No pathologic reactions in either blood and spinal fluid.)

TABLE 1—QUANTITY OF SUGAR IN NORMAL SPINAL FLUID

Author	Percentage of Sugar	
Narwatzki	0.0555	
Cavazzani	0.0188	
Claud Bernard	0.0188	
Mestrezzi	0.048-0.53	(20 cases)
Kopetzki	0.046	(8 cases, Benedict's copper method)
Hopkins	0.060-0.075	(Bang's method)
Jaksch	0.06-0.08	(20 cases)
Schloss and Schroeder	0.054-0.139	(in children, Lewis and Benedict's method)
Kraus and Corneille	0.055-0.110	(22 cases, Lewis and Benedict's method)
Levinson	0.064-0.09	(5 cases)
Our results	0.045-0.095	(9 cases)

TABLE 2—QUANTITY OF SUGAR IN PRESUMABLY NORMAL SPINAL FLUIDS

Hospital Number	Percentage of Sugar	Hospital Number	Percentage of Sugar
14251	0.075	14201	0.067
13555	0.095	14229	0.054
13894	0.045	13936	0.093
13987	0.064	14114	0.039
13936	0.057		

As shown in Table 2, the average amount of sugar in these fluids was 0.069 per cent with a minimum of 0.045 per cent and a maximum of 0.095 per cent.

SUGAR CONTENT OF CEREBROSPINAL FLUID IN DISEASES OF THE CENTRAL NERVOUS SYSTEM

Hopkins¹⁰ states "that a slight increase in the sugar concentration of the blood and spinal fluid occurs in some cases of epilepsy, as it does in other nervous conditions," but he felt that the number of his cases was not sufficient to permit any definite conclusions. "Syphilis frequently reveals lower figures than any other condition with the exception of meningitis."

10 Hopkins. Sugar Content of the Spinal Fluid in Meningitis and Other Diseases, *Am J M Sc* **150** 847, 1915.

Kraus and Corneille¹¹ found that there was no correspondence between the amount of globulin, the number of cells and the sugar content in syphilitic diseases of the central nervous system. He concluded that apparently syphilis does not alter the normal sugar content.

Schloss and Schroeder¹ found normal sugar value in cases of cerebrospinal syphilis, and six cases of various types of idiocy.

Weston¹² examined the spinal fluid of twenty untreated cases of paresis for sugar. These fluids gave 0.0718 per cent as an average against 0.0725 per cent for nine treated cases. Their series of fluids examined for sugar includes twenty cases of dementia praecox, ten of manic-depressive insanity, twenty of paresis, six of epilepsy and nine of imbecility. "Individual differences are quite considerable, especially in the dementia praecox group, but the averages for the different groups show no considerable deviation from normal."

TABLE 3—QUANTITY OF SUGAR IN NERVOUS DISEASES
EXPRESSED IN PERCENTAGE

Disease	No. of Cases	Maximum	Minimum	Average
Syphilitic				
Cerebrospinal syphilis	9	0.107	0.04	0.063
Tabes dorsalis	9	0.094	0.019	0.055
Dementia paralytica	6	0.135	0.054	0.079
Syphilis	3	0.071	0.044	0.064
Nonsyphilitic				
Hemiplegia	3	0.081	0.066	0.073
Disseminated sclerosis	4	0.060	0.029	0.050
Hysteria	4	0.135	0.044	0.080
Neurasthenia	8	0.104	0.05	0.067
Miscellaneous diseases	15	0.107	0.011	0.072
Brain tumor	7	0.095	0.054	0.073
Arteriosclerosis	3	0.093	0.043	0.067

Rieger and Salomon¹³ estimated the sugar in about 130 subjects afflicted with some form of nervous disease. They were unable to confirm Mestrezat's statement¹⁴ that in general paresis the sugar values were uniformly high. They ascribe Mott's results¹⁵—from 0.126 to 0.212 per cent of sugar—from eight miscellaneous psychopathic cases to faulty technique.

Weil¹⁶ also obtained normal values in nervous diseases.

11 Kraus and Corneille. Sugar in Health and Disease, J. Lab. & Clin. Med. **1** 685, 1916.

12 Weston, Paul. Sugar Content of the Blood and Spinal Fluid of Insane Subjects, J. M. Research **35** 199, 1916.

13 Rieger and Salomon. Sugar in Spinal Fluid, Boston M. & S. J. **175** 817, 1916.

14 Mestrezat. L'examen chimique du liquide céphalo-rachidien, Gaz. des hop. **85** 789, 1912, J. de Physiol. et de Path. **14** 504, 1912.

15 Mott. The Cerebrospinal Fluid, Lancet **2** 79, 1910.

16 Weil, M. P. Sugar in the Spinal Fluid, Compt. rend. Soc. de Biol., Paris **81** 436, 1918, Ref. in Brit. M. J. **2** 15, 1918.

From Table 3, compiled from our sugar determinations, it is obvious that although the average sugar value is somewhat higher in general paresis this increase is not sufficient to assume diagnostic significance

SUGAR CONTENT IN MENINGITIS

Schloss and Schroeder¹ claim there is no decrease in the reducing power of the cerebrospinal fluid in meningism, that a large proportion of the cases of tubercular meningitis (twenty-three cases) show a decrease in the sugar content of the cerebrospinal fluid at some stage of the disease, that in a few cases, however, the sugar is normal at all times or diminished but slightly. A decrease, only, is said to be of diagnostic value. The quantity of sugar in epidemic meningitis (eight cases examined) is said to be greatly decreased. Connall³ states that the reducing power in epidemic meningitis usually increased with improvement of the patient. Of the eight cases examined by Schloss and Schroeder, only one showed any increase in sugar though the condition of the patient became worse. A decrease in sugar is said to be present in all miscellaneous types of meningitis.

Kraus and Corneille¹¹ determined the sugar value in two cases of purulent meningitis. The value rose steadily in the case of the patient who recovered, while it fell in the patient who died. They considered the changes in the sugar content of cerebrospinal fluid in purulent meningitis of great value prognostically, a rise being favorable and a fall of unfavorable significance. In this they agree with Hopkins, Schloss and Schroeder, etc. However, they do not confirm the statement of Du Bois and Neal,¹⁷ and Schloss and Schroeder, that sugar is absent in acute purulent meningitis. According to Du Bois and Neal, Fehling's solution was not reduced in 25 per cent. of their tuberculous meningitis cases. Also, in epidemic cerebrospinal meningitis they found it positive or negative according to the severity of the stage of the disease. In meningitis due to other organisms it may be negative but in the early stage it may be positive.

In one of our cases of tubercular meningitis the sugar content was too small to be determined quantitatively. However, Benedict's qualitative reduction test showed the presence of a trace. The patient died a few hours after the puncture. Two consecutive samples from a case of epidemic meningitis showed increasing sugar values. The patient recovered. In all cases, before treatment was instituted, sugar was found below normal.

¹⁷ Du Bois and Neal. Summary of Four Years of Clinical and Bacteriological Experience with Meningitis in New York City, *Am J Dis Child* 9 1, 1915

SUGAR IN DIABETES

According to Mott¹⁵ an increase of the sugar content of the cerebrospinal fluid in diabetes mellitus has long been known. Rieger and Salomon¹³ report values of from 0.134 to 0.256 per cent. The highest sugar value in spinal fluid we encountered in our studies was 0.729 per cent, the blood sugar being 0.747 per cent. This spinal fluid also gave positive tests for acetone and diacetic acid and a carbon dioxide carrying capacity of 13.60 mm Hg while the carbon dioxide carrying capacity of the blood amounted to 27.9 mm.

SUGAR IN OTHER DISEASES

In our series the average sugar content of cerebrospinal fluids obtained from compensated cardiac cases was 0.068 per cent with a maximum of 0.095 and a minimum of 0.045 per cent. The sugar value in two cases of nephritis was slightly above the average normal but not higher than the sugar of individual normal cases. In cystitis, lung disease and thyroid diseases the sugar is normal.

CONCLUSIONS IN REGARD TO SUGAR CONTENT OF CEREBROSPINAL FLUID

1 Normal value is 0.069 per cent (max. 0.095, min. 0.045 per cent).

2 The quantity of sugar is approximately normal in cerebrospinal syphilis, tabes dorsalis, syphilis, hemiplegia, disseminated sclerosis, neurasthenia, brain tumor, arteriosclerosis and other diseases.

TABLE 4 — PERCENTAGE RATIO BETWEEN SUGAR IN PRESUMABLY NORMAL SPINAL FLUID AND SUGAR IN BLOOD OF THE SAME PATIENT

Hospital Number	Case	Blood	Spinal Fluid	Spinal Fluid
				Blood
14055		0.120	0.058	47.8
14381		0.107	0.062	57.4
13555		0.135	0.095	70.3
14114	91	0.118	0.059	49.5
Average normal blood sugar				0.120
Average normal spinal fluid sugar				0.069
Average ratio between 100 × spinal fluid to blood				56.2
(Maximum, 70.3, minimum, 47.8)				

3 The sugar tends to be slightly increased in dementia paralytica and in hysteria. This increase is not sufficient to make it of value in diagnosis.

4 There is a decrease in sugar in tubercular meningitis and epidemic meningitis.

5 In diabetes the sugar quantity is increased proportionately to the blood sugar.

RELATIVE SUGAR CONTENT OF CEREBROSPINAL FLUID AND BLOOD

Schloss and Schroeder made comparative studies of sugar content of blood and cerebrospinal fluid. They write "that it is of great interest that the normal variation in the amount of sugar in the cerebrospinal fluid is very close to that which obtains for blood. It, therefore, seemed of interest to ascertain whether there is any correspondence between the blood sugar and the cerebrospinal fluid sugar in the individual case

TABLE 5 — PERCENTAGE RATIO BETWEEN SPINAL FLUID SUGAR AND BLOOD SUGAR IN VARIOUS DISEASES

Hospital Number	Case	Blood Sugar	Spinal Fluid Sugar	$100 \times \frac{\text{Sp. Fl.}}{\text{Blood}}$	Diagnosis
Syphilitic					
14408	4	0.117	0.040	33.7	Cerebrospinal syphilis*
14444		0.077	0.065	84.3	Cerebrospinal syphilis, spastic paraplegia
14074	7	0.105	0.076	72.1	Cerebrospinal syphilis
15547		0.114	0.034	29.8	Tabes
15508		0.099	0.051	51.5	Tabes
14442	20	0.132	0.087	65.8	Tabes, general paralysis of the insane
14201	16	0.088	0.067	75.0	Tabes
14550	89	0.135	0.091	70.1	Tabes, arthritis
I		0.108	0.096	88.8	Tabes
14538	21	0.35	0.066	49.7	Dementia paralytica
14401	19	0.102	0.135	132.3	Dementia paralytica
14140	18	0.132	0.051	40.9	Dementia paralytica
14329	24	0.105	0.077	72.8	Syphilis
Non-syphilitic					
14086	31	0.114	0.048	42.1	Multiple sclerosis
15528	35	0.100	0.052	52.0	Hysteria
14578	26	1.14	0.090	78.0	Hysteria
15517	42	0.150	0.075	50.0	Neurasthenia
14585	41	0.079	0.077	72.4	Neurasthenia
14062	39	0.12	0.058	47.9	Neurasthenia
14413	42	0.141	0.1035	78.7	Neurasthenia
14055	53	0.12	0.058	47.8	Scleritis (adenopathy)
14318	49	0.107	0.062	57.1	Curvature of spine
14040	57	0.126	0.082	65.0	Narcolepsy
14415	58	0.110	0.079	71.6	Epilepsy
15775	51	0.14	0.102	72.6	Idiocy
14055	53	0.081	0.062	76.0	Paralysis agitans
13715	60	0.203	0.095	47.2	Brain tumor
14531	65	0.142	0.075	52.7	Brain tumor
14055	53	1.20	0.058	47.8	Heart disease (blood taken 4 hours later)
13555	71	0.135	0.095	70.3	Heart disease
15576	69	0.117	0.093	79.5	Heart disease, arteriosclerosis
14021	81	1.25	0.081	70.0	Pyelonephritis†
14251	82	0.097	0.075	77.3	Chronic nephritis, heart disease
D		0.747	0.729	97.6	Diabetes

* Blood taken two days after spinal fluid

† Blood taken three hours after withdrawal of spinal fluid

Accordingly, in ten cases we have made sugar determinations on blood and cerebrospinal fluid which were obtained at the same time. In only two instances were the values similar. These results indicate that although the variations in the blood sugar and the cerebrospinal fluid sugar are practically identical, yet there is no correspondence in the individual case at a given time."

In the series investigated by Weston¹² the ratio of sugar in the spinal fluid to that found in the blood varied from 1 to 1.55 in the epilepsy group to from 1 to 1.72 in the general paralysis group, or

expressed in terms of percentage of spinal fluid to blood, from 64.5 per cent in the epilepsy group 1 to 58.1 per cent in the general paralysis group

Fine and Myers¹⁸ established the ratio of sugar in spinal fluid and blood in fifteen cases of nephritis of various stages of severity, the blood picture varying from normal to pathological. For their series, the sugar concentration of the spinal fluid amounted to about 60 per cent of the quantity present in the blood.

In our series the ratio determined between the sugar content in spinal fluid and blood in four apparently normal cases is shown in Table 4.

The results show that the ratio between the spinal fluid and blood of normal individuals is 56.2, with a maximum of 70.3 and a minimum of 47.8. No constant increase of sugar in spinal fluid is apparent with

TABLE 6—AVERAGE PERCENTAGE RATIO OF SUGAR IN SPINAL FLUID AND BLOOD

	100 × Sp. Fl.	No. of Cases	Maximum	Minimum
	Blood			
Cerebrospinal syphilis	78.3	3		
Tabes dorsalis	63.8	6	88.8	29.8
Dementia paralytica	77.6	3	132.3	40.9
Syphilis	72.8	1		
Hysteria	65.0	2		
Neurasthenia	62.2	4	78.7	47.9
Brain tumor	50.0	2		
Miscellaneous nervous diseases	65.2	6	76.9	47.8
Miscellaneous diseases	63.0	5	79.5	47.8
Diabetes	97.6	1		

an increase of sugar in the blood in normal cases. Similarly no constant increase is found in pathologic cases. Thirty-three determinations of sugar ratios were carried out in various diseases (Table 5).

The ratios, especially in dementia paralytica and tabes, display great inconstancy. To show this still more clearly, Table 6 has been compiled. One can see that in each of the diseases investigated average ratios are obtained more or less approximately normal while in individual cases the results vary widely.

The relative, as well as the absolute, sugar content is highest in diabetes.¹⁹

CREATININ

According to Leopold and Bernhard,²⁰ creatinin varies between 0.7 and 1.5 mg. per 100 c. c. of fluid, the average amount being 0.9 mg. Our determinations of creatinin in presumably normal spinal fluid are recorded in Table 7.

18. Fine and Myers. Comparative Distribution of Urea, Creatinin, Creatin, Uric Acid and Sugar in Blood and Spinal Fluid, *Pr. Soc. Exper. Biol. & M.* 13: 126, 1914.

An analysis of the values for creatinin in eighty-nine cases indicated that the quantity of creatinin in the spinal fluid in various diseases, with the exception of diseases of the kidneys, varies between 0.43 and 2.75 mg per 100 c c, the same range for blood creatinin. The diseases exhibiting high blood creatinin content also exhibit an increase in the creatinin content in the spinal fluid. The highest value was obtained in a case of pyelonephritis. The quantity of creatinin amounted to 5.0 mg.

Four comparative studies of creatinin of normal spinal fluid and blood are recorded in Table 8.

Fine and Myers²¹ determined the ratio of creatinin and found it to be 46 per cent of that of blood in fifteen cases of nephritis with blood pictures varying from normal to markedly abnormal.

Regarding the ratio in pathologic spinal fluid and blood, we find the lowest in a case of cerebrospinal syphilis. However, another case of cerebrospinal syphilis yields a ratio of 130.0. The figures in Tables 9 and 10 show that in the same disease, as well as in different diseases, the ratio is not a constant factor, that at times the creatinin is higher in spinal fluid than in blood, at other times even as little as one-fifth.

Absolute values which are, perhaps, notable are found in one case each of dementia paralytica, hysteria, idiocy, and pyelonephritis.²²

In one case of diabetes, the percentage ratio of creatinin in spinal fluid and blood was 168.7 as against the normal average of 95.5.

CONCLUSIONS

1. The creatinin value in normal spinal fluid varies from 0.45 to 2.20 mg for 100 c c of spinal fluid.

19 Other references bearing on blood sugar are the following:
Ardin, 1912.

Blatters, S. R., and Lederer, M. Analysis of Four Hundred and Twenty-Six Cerebrospinal Fluids from Various Pathologic Conditions, *J. A. M. A.* **60**: 811, 1913.

Jaksch. Klinische Diagnosis Innerer Krankh., Ed. 5, p. 567.

Kiely. Some Recent Studies in the Chemistry of Cerebrospinal Fluid, *J. Lab. & Clin. Med.* **2**: 645, 1916.

Kopetzky. Untersuchungen über die Beziehungen gewisser Gewebsreactionen zur Frühdiagnose und chirurgischen Behandlungsweise der Meningitis, *Ztschr. f. Ohrenh. u. d. Krankh. d. Luftwege* **58**: 1, 1913.

Leopold and Bernhard. Studies in Chemistry of the Spinal Fluid of Children, *Am. J. Dis. Child.* **13**: 34 (Jan.) 1917.

Levinson. Cerebrospinal Fluid in Health and Disease, 1919.

Terotoli. *Ann. d. fac. di med.* **13**: 101, 1913.

Turner. Sugar in Spinal Fluid, *Brit. M. J.* **2**: 60, 1918.

20 Leopold and Bernhard. Studies in the Chemistry of the Spinal Fluid of Children, *Am. J. Dis. Child.* **13**: 34, 1917.

21 Fine and Myers. Comparative Distribution of Urea, Creatinin, Uric Acid and Sugar in Blood and Spinal Fluid, *Pr. Soc. f. Exper. Biol. & Med.* **12**: 126, 1914.

22 Terotoli. *Ann. d. fac. di med.* **13**: 101, 1913.

TABLE 7—QUANTITY OF CREATININ IN PRESUMABLY NORMAL SPINAL FLUID

Hospital Number	Case	Creatinin, Mg in 100 C c	Hospital Number	Case	Creatinin, Mg in 100 C c
14251	70	0 75	14201	76	1 35
13555	71	1 90	14229	78	2 20
13894	72	0 45	13936	79	1 10
13987	74	1 25	14114	91	1 05
13936	75	1 40			

The average of nine determinations was 1 27 mg per 100 cc of fluid, the maximum being 2 20, the minimum 0 45

TABLE 8—PERCENTAGE RATIO OF CREATININ IN NORMAL SPINAL FLUID AND BLOOD

Hospital Number	Blood	Spinal Fluid	100 × Sp Fl Blood
14055	1 64	1 20	75 0
14381	1 70	1 15	67 6
13555	1 10	1 90	172 7
14114	1 55	1 05	66 7

Average ratio of creatinin in spinal fluid and blood is 95 5 (maximum 172 7)

TABLE 9—PERCENTAGE RATIO OF CREATININ IN SPINAL FLUID AND BLOOD IN VARIOUS DISEASES

Hosp No	Case	Blood	Spinal Fluid	100 × Sp Fl Blood	Diagnosis
Syphilitic					
14408	4	2 5	0 55	22 0	Cerebrospinal syphilis
14444		0 45	0 65	130 0	Cerebrospinal syphilis
14074	7	1 75	1 60	91 43	Cerebrospinal syphilis
15547		1 55	1 10	70 3	Tabes
14389	89	1 3	1 25	96 1	Tabes
15808		1 55	1 00	64 5	Tabes
14442	20	<0 45	0 78	>173 0	Tabes
14201	16	1 35	1 35	100 0	Tabes
14338	21	2 55	2 35	92 3	Dementia paralytica
14401	19	1 55	1 70	109 6	Dementia paralytica
14329	24	2 5	1 2	48 0	Syphilis
Nonsyphilitic					
14086	31	2 20	1 25	56 7	Multiple sclerosis
15728	35	1 52	1 10	78 9	Hysteria
14558	36	3 10	2 40	70 9	Hysteria
15817	43	2 05	1 10	53 6	Neurasthenia
14385	41	0 75	0 75	100 0	Neurasthenia
14062	39	1 6	1 2	75 0	Neurasthenia
14413	42	0 58	0 58	100 0	Neurasthenia
14055	53	1 64	1 20	75 0	Sciatica (adenopathy)
14318	49	1 70	1 15	67 6	Curvature of spine
14040	57	1 50	1 50	100 0	Narcolepsy
14435	58	<0 45	0 50	>111 0	Epilepsy
13775	51	2 25	2 25	100 0	Idiocy
14055	53	0 45	0 90	200 0	Paralysis agitans
13715	60	1 90	1 65	81 57	Brain tumor
14534	65	0 45	0 78	162 5	Brain tumor
14055	53	1 64	1 20	73 1	Heart disease
13955	71	1 10	1 90	172 7	Heart disease
15576	69	2 00	1 10	55 0	Heart disease, arteriosclerosis
14021	81	4 00	5 00	125 0	Pyelonephritis*
14251	82	0 75	0 75	100 0	Chronic nephritis, heart disease
14406	84	0 40	0 50	125 0	Chronic cystitis
D		2 70	1 6	168 7	Diabetes

* Blood taken three hours after withdrawal of spinal fluid

2 The ratio between creatinin of normal spinal fluid and blood reveals the existence of a still greater variability than for sugar

3 The ratio between creatinin of pathologic spinal fluid and blood is not sufficiently constant to justify clinical application

UREA

The urea content in the cerebrospinal fluid has been determined previously by many investigators. The results of their studies are shown in Table 11 which indicates the cerebrospinal fluid urea content

TABLE 10—AVERAGE RATIO OF CREATININ IN SPINAL FLUID AND BLOOD OF VARIOUS DISEASES

	100 × Sp Fl	No of	Maxi-	Mini-
	Blood	Cases	mum	mum
Cerebrospinal syphilis	81.1	3	130.0	22.0
Tuberculous	98.7	5	162.5	64.5
Dementia paralytica	100.9	2		
Syphilis	48.0			
Hysteria	74.9	2		
Neurasthenia	82.1	4	100.0	53.6
Brain tumor	122.0	2		
Miscellaneous nervous diseases	109.0	6	200.0	67.6
Pyelonephritis	125.0			
Chronic nephritis	100.0			
Diabetes	168.7			

TABLE 11—UREA CONTENT IN CEREBROSPINAL FLUID

Date	Name	Urea Content in 100 C c
1896	Cavazzani ²⁴	9.8 mg
1896	Thiery ²⁵	13.5 mg
1904	Widal and From ²³	0.015–0.025 %
1906	Fraenkel	0.513 % urea N
1912	Mestrezat	0.06–1 %
	Leopold and Bernhard ²⁶	7.0–13.5 mg
1914	Gumprecht	0.012 % in spinal fluid of cow
1916	Kahn ²⁷	14–33.32 in 100 c c urea N
1916	Tolln	6.25–20.75 urea N
	Personal results	9.87

in presumably normal individuals. Widal and From²³ published urea values up to 43.5 mg per 100 c c in pathologic cases.

Mallard and Foment claim that a notable increase of urea in spinal fluid is always indicative of renal insufficiency and is of valuable aid

²³ Widal and From. *L'urée dans le liquide céphalo-rachidien desBrightiques*, *Gaz. d. hop.* **77** 1182, 1904, *Soc. Biol.* **2** 282, 1904.

²⁴ Cavazzani. *Weiteres über die Cerebrospinalflüssigkeit*, *Centralbl. f. Physiol.* **6** 145, 1896.

²⁵ Thiery (reported by Cavazzani). *Weiteres über die Cerebrospinalflüssigkeit*, *Centralbl. f. Physiol.* **6** 145, 1896.

²⁶ Leopold and Bernhard. *Studies in the Chemistry of the Spinal Fluid of Children*, *Am. J. Dis. Child.* **13** 126 (Feb.) 1917.

²⁷ Kahn and Neal. *Quantitative Chemical Studies in Cerebrospinal Fluid*, *Pr. Soc. Exper. Biol. & Med.* **14** 26, 1916.

in diagnosis Levinson²⁸ determined urea in six pathological fluids, two being cases of meningism, the others cases of psychosis His values range between 3.2 and 9.0 mg per 100 c c

Our findings for urea in presumably normal fluids are tabulated in Table 12 It shows an average value for urea of 9.87 mg per 100 c c of fluid, the extremes being 7.58 mg and 12.75 mg

TABLE 12—QUANTITY OF UREA IN PRESUMABLY NORMAL SPINAL FLUID

Hospital No	Case	Mg per 100 C c	Hospital No	Case	Mg per 100 C c
14251	70	11.25	13936	79	9.28
13936	75	9.00	14114	91	12.75
14201	76	10.80		100	7.58
14229	78	9.00		101	9.36

Average quantity of urea, 9.87 mg per 100 c c

TABLE 13—QUANTITY OF UREA IN THE SPINAL FLUID IN VARIOUS DISEASES

Hosp No	Case	Mg in 100 C c	Diagnosis
Syphilitic			
14384	3	12.25	Cerebrospinal syphilis
14408	4	11.75	Cerebrospinal syphilis
14074		10.5	Cerebrospinal syphilis
14143		11.00	Cerebrospinal syphilis
14203		6.35	Cerebrospinal syphilis
14444		17.5	Cerebrospinal syphilis, spastic paraplegia
14442		19.15	Tabes
15808		18.8	Tabes, prim optic atrophy
13500		13.5	Tabes
14201		10.8	Tabes
14384		12.25	Tabes, arthritis
14168	7	12.0	Dementia paralytica
14140	18	10.75	Dementia paralytica
14401	18	12.50	Dementia paralytica
14442	20	9.50	Dementia paralytica
14338	21	9.45	Dementia paralytica
14329	24	6.3	Syphilis
14233	25	9.5	Syphilis
Nonsyphilitic			
12950	30	6.0	Multiple sclerosis
14086	31	5.26	Multiple sclerosis
13807	40	9.50	Neurasthenia, syphilis
14385	41	12.0	Neurasthenia
14413	42	8.5	Neurasthenia
15817	62	9.96	Brain tumor
13715	63	6.00	Brain tumor
13792	64	6.75	Brain tumor
14534		16.75	Brain tumor
13987	67	12.0	Arteriosclerosis
14168	68	12.0	Arteriosclerosis
15576	69	9.28	Arteriosclerosis
15797	45	10.30	Constitutional inferiority
14449	52	16.75	Paralysis agitans
L		8.6	Psychosis
14229	80	9.0	Heart disease, syphilis
14251		11.25	Chronic nephritis, heart disease
14406		11.00	Chronic cystitis
14106		29.1	Enlargement of prostate

Quantity of Urea in Various Diseases—Tables 13 and 14, respectively, give the figures for the quantity of urea in various diseases As with creatinin, we do not find values appreciable above or below the normal limits for urea in the spinal fluid, however, it is to be noted that in tabes the minimum value is about the same as the upper limit

found for urea in our normal fluids, the other findings being slightly above the normal figures. Whether any importance can be attached to this result or not only further study would reveal. The highest value, 29.1 mg for 100 c c of spinal fluid, was found in a case of enlargement of the prostate.

Ratio Between Urea in the Spinal Fluid and Blood—Cullen and Ellis²⁹ found a difference of less than 2 mg per 1,000 c c in the amounts of urea in the fluid and the blood in 63 per cent of their determinations. The urea content of the fluid varied from 22 to 46 mg and that of the blood serum between 20 and 42 mg. Myers and Fine³⁰ give 88 per cent as the ratio for urea in spinal fluid and blood in nephritis.

TABLE 14—QUANTITY OF UREA IN SPINAL FLUID IN VARIOUS DISEASES

Disease	Average Urea Value, Mg per 100 C c	Cases	Maximum	Minimum
Cerebrospinal syphilis	11.76	6	17.5	6.35
Tuberc	14.9	5	19.5	10.80
Dementia paralytica	10.84	5	12.5	9.50
Syphilis	7.9	2	6.3	9.50
Multiple sclerosis	5.63	2	5.26	6.00
Neurasthenia	10.0	3	12.0	8.50
Brain tumor	9.86	4	16.75	6.00
Arteriosclerosis	11.09	3	12.00	9.30
Miscellaneous diseases	13.71	7	29.1	8.00

TABLE 15—UREA IN NORMAL SPINAL FLUID AND BLOOD

Mg Urea for 100 C c		
Spinal Fluid	Blood	100 × Spinal Fluid
7.58	12.78	Blood
9.36	13.49	61.70
10.61	16.42	78.17
12.75	27.50	64.63
		46.10

Average ratio of urea of normal spinal fluid and blood, 62.15 per cent

In our study we determined the proportion of urea in spinal fluid and blood from four normal individuals (Table 15).

In all pathologic fluids examined all but the group of dementia paralytica exhibit an average ratio above that of normal fluid, cerebrospinal syphilis ranking highest.

CONCLUSIONS

1 The average value for urea in 100 c c of normal spinal fluid is 9.87 mg

²⁹ Cullen and Ellis. The Urea Content of Human Spinal Fluid and Blood, *J Biol Chem* 20 511, 1915

³⁰ Myers and Fine. Comparative Distribution of Urea, Creatinin, Creatin, Uric Acid and Sugar in Blood and Spinal Fluid, *Pr Exper Biol & Med* 13 126, 1916

2 The urea content of cerebrospinal fluid in cerebrospinal syphilis shows a slight increase over the normal

3 The average ratio of urea of normal spinal fluid and blood is 62.15 per cent, this ratio being slightly increased in diseases of cerebrospinal involvement

TABLE 16—PERCENTAGE RATIO OF UREA IN SPINAL FLUID AND BLOOD UNDER VARIOUS DISEASES

Hosp No	Case	Blood	Spinal Fluid	100 × Sp Fl	Diagnosis
				Blood	
Syphilitic					
14408		14.75	11.75	79.6	Cerebrospinal syphilis
14444		9.45	17.5	174.6	Cerebrospinal syphilis, spastic paraplegia
14442	20	13.00	19.50	150.0	Tabes
14201	16	12.00	10.8	90.0	Tabes
14389	89	11.5	12.25	106.5	Tabes, arthritis
L		11.00	8.61	78.3	Tabes
14338	21	15.25	9.45	61.9	Dementia paralytica
14401	19	27.50	12.5	45.5	Dementia paralytica
G		12.00	10.75	89.7	Dementia paralytica
14329	24	12.5	6.3	50.4	Syphilis
Nonsyphilitic					
15817	43	10.66	9.96	93.4	Neurasthenia
14385	41	10.5	12.0	114.2	Neurasthenia
14413	42	12.00	8.5	70.8	Neurasthenia
14055	53	13.50	16.75	124.0	Paralysis agitans
13715	60	11.0	6.0	54.54	Brain tumor
14534	65	10.75	16.75	155.8	Brain tumor
15576	69	11.50	9.28	80.7	Heart disease, arteriosclerosis
14251	82	11.75	11.25	95.7	Chronic nephritis, heart disease
14406	84	11.75	11.00	93.6	Cystitis
D		17.45	17.96	102.5	Diabetes

TABLE 17—UREA IN SPINAL FLUID AND BLOOD IN OTHER DISEASES

Disease	Average Percentage of Urea in Spinal Fluid and Blood
Cerebrospinal syphilis	127.1
Tabes	84.9
Dementia paralytica	65.7
Neurasthenia	92.8
Brain tumor	105.17

ACID-BASE EQUILIBRIUM IN CEREBROSPINAL FLUID (RESULTS ON TITRATION)

A number of studies have been made on the degree of alkalinity of the spinal fluid. Cavazzani³¹ found the reaction neutral in two cases of hydrocephalus. Concetti³² examined seven fluids of which three were alkaline, four weakly alkaline. Turner found the spinal fluid to be alkaline to litmus and acid toward phenolsulphonephthalein. He also noticed a change in alkalinity on standing. Kopetzki is of the

³¹ Cavazzani, E. *Weiberes über die Cerebrospinalflüssigkeit*, *Centralbl f Physiol* **10** 145, 1896.

³² Concetti. *Chemische Untersuchungen über die Hydrocephalusflüssigkeit von Kindern*, *Arch f Kinderh* **24** 161, 1898.

TABLE 18—DETERMINATION OF SUGAR, CREATININ AND UREA
IN PATHOLOGIC FLUIDS

Hosp No	Case	Sugar	Creatinin	Urea	Diagnosis
Syphilitic					
15666	1	0.068	1.5		Cerebrospinal syphilis
14177	2	0.1065	1.4		Cerebrospinal syphilis
14384	3	0.091	1.25	12.25	Cerebrospinal syphilis
14408	4	0.040	0.55	11.75	Cerebrospinal syphilis
13189	5	0.029	1.4		Cerebrospinal syphilis
13946	6	0.01	0.95		Cerebrospinal syphilis
14074	7	0.076	1.75	10.5	Cerebrospinal syphilis
14143	8	0.069	1.4	11.0	Cerebrospinal syphilis
14203	9	0.040	1.1	6.35	Cerebrospinal syphilis
14444		0.065	0.65	17.5	Cerebrospinal syphilis, spastic paraplegia
13218	10	0.067	1.70		Tabs
14257	11	0.054	2.50		Tabs
14142	12	0.087	0.65	10.15	Tabs
15547	13	0.031	1.10		Tabs, neuritis
15808	14	0.032	1.10	18.8	Tabs, prim optic atrophy, fluid withdrawn for diagnosis
15808	14	0.019	1.00		Ten days later withdrawn for treatment
13009	15	0.047	1.73	13.5	Tabs
14201	16	0.067	1.35	10.8	Tabs
14284	16	0.094	1.25	12.25	Tabs, arthritis
14168	17	0.065	1.55	12.0	Dementia paralytica
14140	18	0.051	2.75	10.75	Dementia paralytica
14401	19	1.35	1.70	12.50	Dementia paralytica
14442	20	0.087	0.65	9.5	Dementia paralytica
14378	21	0.065	2.53	9.45	Dementia paralytica
14168	22	0.068	1.55		Dementia paralytica, cardiac hypertrophy
13807	23	0.044	2.1		Syphilis, hysteria
14320	24	0.076	1.20	6.3	Syphilis
14233	25	0.071	1.9	9.5	Syphilis
Nonsyphilitic					
13377	26	0.081	1.90		Right hemiplegia due to cerebral hemorrhage
13161	27	0.066	1.58		Right hemiplegia
13710	28	0.071	1.60		Right hemiplegia, embolic
13189	29	0.029	1.4		Multiple sclerosis
12070	30	0.058	1.60	6.0	Multiple sclerosis
14056	31	0.051	1.25	5.26	Multiple sclerosis
15649	32	0.060	1.10		Multiple sclerosis
13481	33	0.135	0.51		Hysteria
13897	34	0.044	2.10		Hysteria, syphilis
15728	35	0.052	1.10		Hysteria
14558	36	0.090	2.40		Hysteria
13429	37	0.066	1.58		Neurasthenia
13650	38	0.052	4.0		Neurasthenia, obesity
14062	39	0.058	1.20		Neurasthenia
13837	40	0.071	1.90	9.50	Neurasthenia
14385	41	0.057	0.75	12.0	Neurasthenia
14413	42	0.104	0.58	8.5	Neurasthenia
15817	43	0.075	1.10		Neurasthenia
13751	44	0.05	1.1		Neurasthenia, cystitis, acute
15707	45	0.103	0.43	10.30	Constitutional inferiority
14177	46	0.107	1.30		Constitutional inferiority and cerebrospinal syphilis
	47	0.064	1.60		Constitutional inferiority
13729	48	0.049	1.38		Cervical ribs
14318	49	0.062	1.15		Curvature of spine
13959	50	0.091	1.53		Fracture of spine
13775	51	0.411	2.25		Idiocy
14449	52	0.062	0.90	16.75	Paralysis agitans
11055	53	0.051	1.05		Sciatica (adenopathy)
12681	54	0.076	2.55		Convulsive fits
13190	55	0.065	1.39		Epilepsy, Jacksonian
13792	56	0.064	1.93		Epilepsy
14040	57	0.082	1.5		Narcolepsy
14435	58	0.079	0.50		Epilepsy (syphilitic)
	59	0.059	2.60		Chronic leptomenigitis, pernicious anemia
L					
13715	60	0.096	1.0	8.6	Psychosis
13715	60	0.071	1.35		Brain tumor
13702	61	0.095	1.65	2 wks later	Brain tumor
13702	61	0.057	1.50		Brain tumor
15817	62	0.075	1.10	9.96	Brain tumor
13715	63	0.095	1.65	6.00	Brain tumor
13792	64	0.057	1.50	6.75	Brain tumor
14534	65	0.075	0.78	16.75	Brain tumor

TABLE 18—DETERMINATION OF SUGAR, CREATININ AND UREA
IN PATHOLOGIC FLUIDS—(Continued)

Hosp No	Case	Sugar	Creatinin	Urea	Diagnosis
13476	66	0.043	2.40		Arteriosclerosis
13987	67	0.064	1.25	1.20	Arteriosclerosis
14168	68		1.55	12.0	Arteriosclerosis
15576	69	0.093	1.10	9.28	Arteriosclerosis
14251	70	0.075	0.75	11.25	Valvular disease
13555	71	0.095	1.9		Valvular disease, chronic myocarditis, chronic cardiac disease
13894	72	0.045	1.10		Valvular disease, chronic cardiac acute arthritis
14021	73	0.084	5.00		Double lobar pneumonia, pyelonephritis
13987	74	0.064	1.25		Chronic endocarditis, cardiac hypertrophy
13936	75	0.057	1.40	9.00	Card hypertrophy, valv disease
14201	76	0.067	1.35	10.8	Chronic valvular disease
14168	77	0.0655	1.55		Heart disease, paralysis gen
14229	78	0.054	2.2	9.0	Chronic valvular disease
13936	79	0.093	1.10	9.28	Chronic valvular disease
14229	80	0.054	2.20	9.0	Syphilis, heart disease
14021	81	0.084	5.0		Pyelonephritis
14251	82	0.075	1.10	11.25	Chronic nephritis, heart disease
13511	83	0.050	1.40		Cystitis neurasthenia
14406	84	0.075	0.50	11.0	Chronic cystitis
13569	35	0.059	0.60		Gangrenous cystitis
13461	89	0.040	1.85		Emphysema
14332	87	0.017	1.60		Pulmonary tuberculosis
14021	73	0.084	5.0		Double lobar pneumonia, pyelonephritis
14231	88	0.055	1.05		Hyperthyroidism
16625	89	0.060	0.70		Myxedema
14037	90	0.088	2.1		Chronic amygdalitis
14114	91	0.059	1.05	12.75	Amygdalitis
14231	88	0.055	1.05		Arthritis, hyperthyroidism
14384	89	0.094	1.25	12.25	Arthritis, tabes
13569	90	0.059	2.60		Pernicious anemia
	92	0.066	1.5		Secondary anemia
14106	94	0.084	2.7	29.1	Enlargement of prostate

opinion that all normal fluids are neutral to litmus, that their P_H is below 8. Von Jaksch found the alkalinity to equal 20 c cm of n/10 acid. Mott considers the alkalinity of spinal fluid of equal ion concentration as a 1 per cent solution of sodium hydroxid. Kafka also found that on an average 20 c c tenth normal hydrochloric acid were necessary to neutralize 100 c c fluid. He used cochineal as an indicator. Bisgaard,³³ using the gas chain method, determined the hydrogen ion concentration in two cerebrospinal fluids and gives the P_H value as more than 8.1. Polanyi³⁴ used the compensation method with the Farkas Szilisch electrode in a case of hydrocephalus and he gives the P_H 9.84 x 10⁻¹¹. Hurwitz and Trauter³⁵ applying the Levy-Rowntree-Marriott method found the P_H equal to 8.15-8.30. These are the results of examinations of fluid from nineteen normal and twenty-eight

33 Bisgaard, A. Untersuchungen über die Eiweiss- und Stickstoffverhältnisse der Cerebrospinalflüssigkeit sowie über die p_H derselben, *Biochem Ztschr*, **58** 1, 1914.

34 Polanyi, U. Beiträge zur Chemie der Hydrocephalusflüssigkeit, *Biochem Ztschr* **34** 205, 1911.

35 Hurwitz, S. H., and Trauter, C. L. On the Reaction of Cerebrospinal Fluid, *Arch Int Med* **17** 828 (June) 1916.

syphilitic patients Weston³⁶ examined 105 fluids from individuals mentally deficient The p_H varied from 7.9 to 8.3, average 8.12 The results do not differ from those obtained by Hurwitz and Trauter and are in accord with the statement of Mott³⁷ that the reaction of the cerebrospinal fluid varies only slightly in different conditions

McClendon,³⁸ using microtitration with dibromorthocresolsulphonephthalein as an indicator found that in a few cases (no reference to disease) 0.027-0.0275 c c normal hydrochloric acid, for two cases of diabetes, 0.015 and 0.030 c c hydrochloric acid respectively Levinson³⁹ arrives at the conclusion that the P_H of normal cerebrospinal fluid is the same as that of blood, that it also ranges between 7.4 and 7.6 immediately after being removed from the body

TABLE 19—ACID-BASE EQUILIBRIUM IN CEREBROSPINAL FLUID

Date	Name	Findings
1896	Cavazzani	15.7 c c sulphuric acid
1898	Concetti	3 fluids alkaline, 4 weakly acid
1910	Turner	Alkaline to litmus, acid to phenolsulphonephthalein
1911	Polanyi	$0.084 \times 10''$
1913	Kopetzky	Neutral to litmus, >8.0
	Von Jaksch	20 c c tenth normal acid
	Mott	0.1% sodium hydroxide
	Kafka	20 c c tenth normal hydrochloric acid
1913-14	Blagaard	<8.1
1916	Hurwitz	7.26
1916	Levinson	2.0-2.4 hundredth normal sulphuric acid (methylred)
		1.15-2.66 hundredth normal sulphuric acid (methylorange)
1917	Weston	8.12
1917	Felton	7.8 (normal and pathologic)
1918	McClendon	0.027-0.0275 normal hydrochloric acid
	Levinson	7.4 - 7.6

Results Obtained in this Investigation by Titration Method— McClendon's method was used To 1 c c spinal fluid in a small porcelain dish two drops of aqueous solution of dibromorthocresolsulphonephthalein were added and tenth normal hydrochloric acid was added from a microburet under agitation until the color changed to yellow The average for normal spinal fluid from cardiac cases with well compensated valvular disease was 0.0249 c c normal hydrochloric acid Owing to difficulty in finding volunteers for the study of normal cerebrospinal fluid cardiac cases with well compensated valvular lesions without evidence of acidosis were accepted as exhibiting normal values Ten such cases yielded an average value of 0.0249 c c normal hydro-

36 Weston, P. G. The Reaction of the Cerebrospinal Fluid, *J. M. Research* **35** 367, 1917

37 Mott Oliver Sharpey Lectures on the Cerebrospinal Fluid, *Lancet* **2** 1, 79, 1910

38 McClendon, J. F. Detection of Acidosis, *J. A. M. A.* **70** 977 (April 6) 1918

39 Levinson Studies in Alkalinity of Cerebrospinal Fluid, *Arch. Pediat.* **23** 241, 247, 1916

chloric acid (the maximum being 0.0263 and minimum 0.0211) Practically all the groups of cases studied yielded values closely approximating normal. The results of these studies will be found in Table 20.

In eighty-four cases studied, the only values below 0.0211 c.c. normal hydrochloric acid were encountered in diabetes, 0.0185, in pyelonephritis, 0.0110, in cerebrospinal syphilis, 0.0146, in syphilis, 0.0149, in leptomeningitis, 0.0164, in brain tumor, 0.019, and in gangrenous cystitis, 0.0164. In cases of diabetes and pyelonephritis marked acidosis was present, while in the other five cases no explanation for the low values was apparent.

TABLE 20—RESULTS OBTAINED BY TITRATION METHOD

No. of Cases	Diagnosis	C c Nor- mal HCl	Maxi- mum	Mini- mum
Syphilitic				
8	Cerebrospinal syphilis	0.02863	0.0354	0.0146
6	Tabes dorsalis	0.0277	0.0294	0.0260
7	Dementia paralytica	0.0265	0.0282	0.0251
4	Syphilis	0.0253	0.0286	0.0149
Nonsyphilitic				
3	Hemiplegia	0.0250	0.0265	0.0235
4	Multiple sclerosis	0.0224	0.0280	0.0146
4	Hysteria	0.0238	0.0290	0.0149
7	Neurasthenia	0.0263	0.0286	0.0233
12	Miscellaneous nervous diseases	0.0246	0.0354	0.0164
7	Brain tumors	0.0260	0.0293	0.019
4	Arteriosclerosis	0.0261	0.0282	0.0229
3	Cystitis	0.0254	0.0280	0.0164
1	Diabetes	0.0185		
1	Pyelonephritis	0.0110		

STUDIES OF ACID-BASE EQUILIBRIUM OF CEREBROSPINAL FLUID

(a) *Alkaline Reserve*—The first reference to the carbon dioxide content of spinal fluid is that of Mott³⁷. The following tabulation is taken from Mott:

	Cerebrospinal Fluid Yield Carbon Dioxide per Cent	Lymph by Volume, Carbon Dioxide per Cent
By vacuum and heating	10	46
By acid and heating in vacuum	50	50
Difference, repr. CO ₂ in stable combination	40	4

Felton, Hussey and Bayne-Jones⁴⁰ discuss Mott's findings as follows: "Mott has interpreted these findings to indicate that the carbon dioxide is in more stable combination in the spinal fluid than in the blood." A careful analysis of his procedure, however, throws doubt on the validity of these conclusions. They continue, "the final factor in the relationship between the reaction of the blood and spinal fluid must depend largely on the possibility of an interchange of free hydrogen ions between the two fluids. In view of the great diffusibility of the hydrogen-ion concentration, it does not seem likely that the choroid plexus and the vascular meninges would obstruct the passage of this ion from the

40 Felton, L. D., Hussey, R. G., and Bayne-Jones. Reaction of Cerebrospinal Fluid. Preliminary Report Arch Int Med 19: 1085 (June) 1917.

blood into the spinal fluid. Since, therefore, the previous studies on the physical and chemical constitution of the spinal fluid and blood lead to the supposition that the reaction of these fluids should be approximately equal, any reports which point to an opposite conclusion require investigation."

TABLE 21—ALKALINE RESERVE OF PATHOLOGIC SPINAL FLUIDS

No. of Cases	Diagnosis	Volume per Cent
4	Diseases of nervous system	53.1
1	Cerebrospinal syphilis	56.7
5	Tabes	53.1
4	Dementia paralytica	46.9
1	Syphilis	51.85
2		53.2
2	Neurasthenia	54.5
4	Brain tumor	51.2
1	Narcolepsy	28.15
1	Pyelonephritis	23.75
6	Diabetes	28.28
	Acidosis	13.60

TABLE 22—ACID-BASE EQUILIBRIUM

Hospital Number	Case	C c Normal HCl	Alkaline Reserve	Diagnosis
Syphilitic				
14143	8	0.0288	56.7	Cerebrospinal syphilis
14237	11	0.0267	40.9	Tabes
15347	13	0.025	55.8	Tabes, neuritis
15808	14	0.029	59.8	Tabes, atrophy
13500	15	0.0287	54.5	Tabes
14201	16		64.8	Tabes
14168	17	0.0282	56.6	Dementia paralytica
14031	22	0.0256	35.2	Dementia paralytica
14338	27	0.0253	51.2	Dementia paralytica
14168	22	0.0251	45.0	Dementia paralytica, cardiac hypertrophy
14320*	24	0.026	51.85	Syphilis
Nonsyphilitic				
14086	31	0.0237	52.6	Diss. sclerosis
15649	32	0.028	53.8	Diss. sclerosis
14062	39	0.0238	53.1	Neurasthenia
15517	43	0.019	55.9	Neurasthenia
15797	45		45.0	Constitutional inferiority
14318	49	0.0298	51.9	Neuritis of sciatic nerve
14040	57	0.028	28.15	Narcolepsy
13792	61	0.0241	51.9	Brain tumor
15817	62	0.019	55.9	Brain tumor
13715	63	0.0285	46.0	Brain tumor
13792	64	0.024	51.19	Brain tumor
13987	67	0.0229		Arteriosclerosis
14021	73	0.0109	23.75	Pyelonephritis
13987	74	0.0229		
13936	75	0.0283		
14168	77	0.0282	56.6	Paralysis gen.
14220†	78	0.0211		
14021		0.011		Pyelonephritis
14037	90	0.0251	45.1	Perniciolous anemia
D		19.48		Diabetes
18214		30.69		Diabetes
18378		35.06		Diabetes
18209		35.06		Diabetes
		35.52		Diabetes

* Alveolar CO₂, 35.4

† Alveolar CO₂, 40.13 (a comparison of the alkaline reserve of the spinal fluid and blood)

Levinson determined the alkaline reserve or the carbon dioxide combining power of the cerebrospinal fluid by the Van Slyke method. He shows that the alkaline reserve in nine pathologic (but nonmeningi-

tic) fluids varies between 45.7 and 63.0 volumes per cent at 0 temperature, and at 760 barometric pressure

In our investigation, Van Slyke's method was also used on nonpathologic and pathologic spinal fluids. The values for normal fluids (four in number) varied between 46.5 and 61.7, with an average of 53.1 c.c. carbon dioxide in 100 c.c. fluid. The values in thirty pathologic fluids are recorded in Table 21.

Only eight values below 45 volume per cent were encountered in this series, one case each of dementia paralytica, narcolepsy, tabes, pyelonephritis and five cases of diabetes mellitus (19.78, 30.69, 35.06, 35.06, 13.6 c.c., respectively). In five cases of this series both the titration and alkaline reserve method were utilized. Van Slyke's method alone showed a decrease of the carbon dioxide combining power in three cases, while in the other two, diabetes and pyelonephritis, increased acidity was demonstrated by both methods.

TABLE 23—ALKALINE RESERVE IN SPINAL FLUID AND BLOOD

Hospital No	Case	CO ₂ Volume per Cent		100 × Sp. Fl.	Diagnosis
		Spinal Fluid	Blood	Blood	
A	14086	52.6	64.2	81.9	Diss. sclerosis
	14829*	51.85	59.0	87.9	Syphilis
	14318	51.9	57.5	90.3	Neuritis of sciatic nerve
	14201	64.8	71.1	91.1	Tabes
	16440	48.7	50.6	96.2	Chorea
B	14021	23.75	18.5	128.3	Pyelonephritis
	D†	19.78	27.9	70.9	Diabetes
	18214	30.69	31.92	96.1	Diabetes
	18378	35.06	34.47	101.7	Diabetes
	18299	35.06	38.51	93.4	Diabetes
		35.52	31.9	111.27	Diabetes

* CO₂ alveolar air = 35.4, patient breathed very lightly

† Blood and spinal fluid were taken right after death, alkaline reserve of blood before death was 27 c.c.

The comparative results of the two methods are shown in Table 22.

In Table 23 are shown the comparative results of a study of the acid base equilibrium of the cerebrospinal fluid and the blood.

In series A, the blood throughout yielded normal values and consequently the acid base equilibrium of this group of cases closely approximates normal in all probability. In series B, on the other hand, acidosis was present in every instance. The presence is indicated in the values found both in the blood and the cerebrospinal fluid.⁴¹

CONCLUSIONS

Under normal conditions the carbon dioxide carrying capacity of the cerebrospinal fluid is somewhat lower than that of blood, while in acidosis it is greater in some instances at least. Whether or not this indicates the operation of a mechanism for the protection of the nervous system is not yet clear.

FERMENTS

The ferments of the blood have played a very minor rôle in medical diagnosis. Lipase has received the most attention. Whipple⁴² and his co-workers made quantitative studies of the lipase in the blood and showed that it was definitely increased in the presence of deficient liver function. They considered an increase of diagnostic significance in eclampsia. It may also be increased in jaundice, pneumonia, peritonitis, leukemias, various infections, in atrophy or necrosis of the liver. Its value is lowered in cirrhosis of the liver. The ferment activity of the spinal fluid has received still less attention. As far as we are aware it has never assumed any clinical importance.

Lipase in Spinal Fluid—Clark and Garnier⁴³ in an article published in 1909 believed lipase to be present in cerebrospinal fluids. Pribram⁴⁴ made a test for lipase in spinal fluid from a patient with a cerebral tumor, the test was negative. Galletta, however, claims to have found lipase in three of seven cases, while Clark found none. Kafka⁴⁵ writes that even nonpathologic spinal fluids may contain diastatic, antitryptic and lypolitic ferments, though much less than the blood serum and the quantity is apparently independent of diseases other than those of the nervous system. In diseases of the central nervous system these ferments are present in increased numbers. Citron⁴⁶ and Klinkert state that the spinal fluid of some paralytic cases with a positive Wassermann exhibits fat splitting properties.

In our investigations of lipase, Whipple's quantitative method was used.

41 The following references also bear on the subject of acid-base equilibrium

Fine, M. S., and Myers, V. C. Comparative Distribution of Urea, Creatinin, Uric Acid and Sugar in the Blood and Spinal Fluid, *J Biol Chem* **37** 239, 1919

Folin, O. On the Determination of Creatinin and Creatin in Blood, Milk and Tissues, *J Biol Chem* **17** 475, 1914

Leopold and Bernhard. Studies in Chemistry of the Spinal Fluid in Children, *Am J Dis Child* **13** 34, 1917

Marriott, W. McK. A Method for the Determination of the Alkali Reserve of the Blood Plasma, *Arch Int Med* **17** 840 (June) 1916

Woods, Allan C. *Arch Int Med* **16** 620 (Oct) 1915

Zdarek, E. Ein Beitrag zur Kenntniss der Cerebrospinalflüssigkeit, *Ztschr f physiol Chem* **35** 201, 1902

42 Whipple, G. H., Mason, V. R., and Peightal, T. C. Tests for Hepatic Function and Disease Under Experimental Conditions, *Johns Hopkins Hosp Bull* **24** 207, 343, 1913

43 Garnier. *Compt rend d 1 soc de biol*, **55** 1389, 1903

44 Pribram. Studien über das lipolitische ferment, *Centralbl f inn Med* **31** 4, 1910

45 Kafka. Zur Biologie der Liquor cerebrospinal. *Mitt d Hamb Staatsirrenanstalt* **13** 47

46 Citron J., and Klinkert, D. Ueber den biologischen Nachweis liporder Substanzen, *Berl klin Wchnschr* **47** 1614, 1910

Determination of Lipase—Four tubes were used To each at least 1 c c spinal fluid was added and made up to 5 c c with distilled water In some cases as much as 5 c c spinal fluid was used To two of them 26 c c ethylbutyrate was added, the other two tubes being controls Toluol (0.3 c c for each tube) was used as preservative The tubes were incubated at 37 C for from eighteen to twenty-four hours After cooling, the fluids were titrated with 0.1 normal hydrochloric acid, using azolitmin as an indicator and 1 c c micropipets as burets The results are tabulated in Table 24

TABLE 24—LIPASE CONTENT IN SPINAL FLUID OF VARIOUS DISEASES

Number	Diagnosis	O c 0.01 HCl in Sp Fl	Number	Control, C c 0.01 HCl	Number of Determi- nation
17638	Paresis	3.7	2	3.73	2
17751	Paresis	4.7	2	4.95	2
17638	Paresis	5.24	2	5.04	2
N	Cerebrospinal syphilis	4.0	3	5.04	2
B	Cerebrospinal syphilis	4.9	1	4.55	1
17848	Cerebrospinal syphilis	3.62	2	3.65	2
McD	Cerebrospinal syphilis	3.96	1	3.96	2
S	Cerebrospinal syphilis	4.24	2	4.26	2
17786	Tabes	4.32	2	4.47	2
17811	Tabes	4.92	2	4.98	2
17749	Tabes	3.30	2	3.41	2
17992	Secondary syphilis	3.20	2	3.28	2
17663	Tertiary syphilis	4.1	1	3.98	2
17779	Syphilis	4.13	2	4.11	2
17885	Congenital syphilis	4.29	2	4.08	2
17761	Blood Wassermann positive, spinal fluid negative	4.17	2	4.18	2
17691	Friedrich's ataxia	4.17	1	4.17	2
17627	Crutch paralysis	4.1	1	4.03	2
17611	Hysteria	4.24	2	4.8	2
17844	Hemiplegia	4.43	2	4.55	2
17675	Nonsyphilitic	4.62	2	4.47	2
17675	Nonsyphilitic	4.03	2	4.05	2
17729	Aneurysm	5.36	2	5.42	2
17760	Osteomyelitis	3.25	2	3.52	2
17777	Diagnosis deferred	4.33	3	3.60	1
17879		2.8	2	2.8	2

CONCLUSIONS

In only two fluids among twenty-six was the presence of any lipase even suggested

Diastase—Cavazzani⁴⁷ found diastase present in the spinal fluid of a dog Panzer and Lewandowsky obtained negative results in two cases of hydrocephalus Lutkye found it present in one case of cerebrospinal meningitis Kafka⁴⁸ noted higher amounts in paresis and in dementia praecox

The method of Myers and Kilian⁴⁹ was used in making our determination

⁴⁷ Cavazzani: Weiteres über die Cerebrospinalflüssigkeit, *Centralbl f Physiol* **10** 145, 1896

⁴⁸ Kafka, V: Mitt d Hamb Staatsirrenanstalt **13** 47

⁴⁹ Myers, V C, and Kilian, J A: Studies on Animal Diastase, *J Biol Chem* **29** 179, 1917

Four 1 c c samples of cerebrospinal fluid are taken, two being used as controls. The control tubes are made up to 5 c c with distilled water, the other tubes to 4 c c and all four tubes are then placed in a water bath of 40 C. As soon as the contents are brought to this temperature, 1 c c of 1 per cent soluble starch solution is added to the tubes to be tested, and after mixing the tubes are incubated at 40 C for exactly thirty minutes. Subsequently, Benedict's quantitative blood sugar method is applied to all four tubes. Of thirty determinations all but two

TABLE 25—DETERMINATION OF DIASTASE IN SPINAL FLUID
OF VARIOUS DISEASES

Number	Sugar per Cent Plus Starch	Sugar per Cent Without Starch	Diastase Sugar per Cent	Diagnosis
City Hosp	0 000	0 000	0 000	Tuberculous meningitis
City Hosp	0 000	0 000	0 000	Epidemic meningitis
M O H	0 598	0 059	0 0008	
S O	0 0052	0 0029	0 0023	Epidemic meningitis
17865	0 0483		0 0023	Fracture case
M L	0 0161	0 0130	0 0034	Multiple sclerosis
18072	0 0626	0 0574	0 052	Tabs
18022	0 0570	0 0526	0 0051	Chronic arthritis
18102	0 0573	0 0109	0 0056	
18131	0 0603	0 5360 536	0 0067	Petit mal
18079	0 0555	0 0482	0 0073	Neurasthenia
18151	0 117	0 109	0 008	Epilepsy
17729	0 0544	0 0158	0 0086	Aneurysm, syphilis
P	0 039	0 030	0 009	Paresis
18030	0 0615	0 052	0 0097	Nephritis
S O	0 0165	0 0568	0 0103	Epidemic meningitis
Y	0 060	0 048	0 012	Encephalitis
R	0 063	0 049	0 011	Epidemic meningitis
17892	0 0799	0 0054	0 0113	Jaundice
18023	0 083	0 0628	0 0202	
18935	0 080	0 057	0 033	Multiple neuritis
D	0 0962	0 0790	0 0232	Tabs, angina pectoris
17963	0 103	0 0736	0 029	Spastic torticollis
17952	0 100	0 07	0 031	Probable lethargic encephalitis
17982	0 001	0 0696	0 0314	Meniere's disease
17982	0 1011	0 0696	0 0388	
17568	0 100	0 0624	0 0376	Normal
18177	0 143	0 098	0 015	Diagnosis deferred

fluids showed the presence of diastase. The highest amount was 0 0564 per cent in a case of spastic torticollis. One case of paresis gave a low amount of 0 009. Fluids from cases of meningitis gave low readings, 0 0023, 0 0103, 0 014, and 0 000 in two cases.

Table 25 shows definitely that there is no regularity between the diastase content and the quantity of sugar.

Comparing the diastatic activity of normal spinal fluid with that of blood (Myers and Kilhan's value for normal diastatic activity, 0 0825 per cent being accepted as the basis for normal blood activity) we find that the diastatic activity of cerebrospinal fluid is 21 9 per cent of that of the blood.⁵⁰

⁵⁰ Leschke and Pincussohn. Untersuchungen über die Fermente der Cerebrospinalflüssigkeit des Menschen, Deutsch med Wchnschr 43 8, 1917.

Trypsin—Panzer,⁵¹ Leopold and Bernhard,⁵² and Schuetz⁵³ all found no proteolytic enzyme in spinal fluid Link and Pollak⁵⁴ report finding a peptolytic enzyme in normal cerebrospinal fluid (Index = 1, rarely = 0, the index for normal blood varying from 10 to 20) In three cases of hydrocephalus and one case of spina bifida examined for the presence of pepsin or trypsin, Halliburton⁵⁵ found no trace of either Miller found no proteolytic enzyme or antienzyme in normal cerebrospinal fluid Douchez⁵⁶ thought the tryptic ferment present, Kafka⁵⁷ thought it absent

We added a 0.5 per cent solution of casein in 0.5 per cent solution of sodium bicarbonate, and incubated the fluids for twenty-four hours at 37 C Subsequent addition of four drops of 10 per cent acetic acid produced a cloud in every spinal fluid Even using as small a quantity as 0.25 c c casein solution, and as large a quantity of cerebrospinal fluid as 4 c c the results were still negative Change of the p_H of the test solution by adding 0.5 per cent sodium bicarbonate did not alter the results Thus our experiments for trypsin showed the absence of this enzyme in the spinal fluid

SPECIFIC GRAVITY

Specific gravity determinations were first made on the spinal fluid of the dog and the calf Cavazzani⁵⁸ in the one specimen he analyzed found the specific gravity 1.009 (in the evening) and 1.012 (in the morning) Narvatzki⁵⁹ determined the specific gravity in 12 specimens using a pycnometer at a temperature range of from 22 to 26 C He found the specific gravity varied from 1.0075 to 1.0080 The determination of specific gravity of human spinal fluid has been carried out by Landau and Halpern⁶⁰ in twenty-two cases They neglect, however,

51 Panzer Zur Kenntniss der Cerebrospinalflüssigkeit, Wien klin Wchnschr **11** 805, 1899

52 Leopold, T. S., and Bernhard Chemistry of Spinal Fluid of Children Am J Dis Child **13** 34 (Jan) 1917

53 Schuetz Proteolytische Fermente in Cerebrospinalflüssigkeit, Centralbl f inn Med **23** 1161, 1902

54 Link, R., and Pollak, L. Ueber das Vorkommen von peptolytischen Fermenten in Exudaten und dessen diagnostische Bedeutung, Deutsch Arch f klin Med **109** 350, 1912

55 Halliburton, W. D. Cerebrospinal Fluid, J Physiol **10** 232, 1889

56 Douchez, A. R. Proteolytic Enzymes and Anti-Enzymes of Normal and Pathological Cerebrospinal Fluids, J Exper M **11** 718, 1909

57 Kafka, V. Zur Biologie des Liquor cerebrospinalis, Neurol Centralbl **31** 627, 1912, Mitt d Hamb Staatsirrenanstalt **13** 47

58 Cavazzani Weiteres über die Cerebrospinalflüssigkeit, Centralbl f Physiol No 6 145 1896

59 Narvatzki Zur Kenntniss der Cerebrospinalflüssigkeit, Ztschr f Physiol Chem **23** 532, 1897

60 Landau and Halpern Beitrag zur Chemie der Cerebrospinalflüssigkeit, Biochem Ztschr **9** 72, 1908

to mention all details pertaining to the accuracy of their determinations. Hence their results (specific gravity, from 1.005 to 1.009) are open to criticism.

Table 26 represents the average value for specific gravity of cerebrospinal fluid as given by a number of investigators.

In our work the specific gravity was estimated with a capillary pycnometer of about 2 c.c. capacity devised by Ostwald, and the specific gravity of water was determined simultaneously with that of spinal fluid to avoid a possible error due to the changes of temperature. Twenty-nine cerebrospinal fluids, normal and pathologic, were examined and the specific gravity found practically alike in all cases. The lowest reading (in a case of leukemia) was 1.0080, three cases (sarcoma

TABLE 26—SPECIFIC GRAVITY OF SPINAL FLUID

Ch. Riebet	1.006
Ch. Robin	1.005
Tolson and Lenoble ⁶¹	1.007
Lassaigne	1.008
Mareet	1.006
Cheritler	1.002
Widal and Stearns	1.004
Quinke	1.006-1.007
Our results	1.0086

of base of brain, dementia paralytica and pyelonephritis, respectively) had a specific gravity of 1.0088. Twelve of the twenty-nine fluids had a specific gravity of 1.0086, which also was the average of all determinations.

Comparison of Specific Gravity of Cerebrospinal Fluid and Blood—In adult human beings the specific gravity of blood serum ranges between 1.045 and 1.075, with an average of 1.058 (Hammersten)⁶². Taking for comparison 1.058 as specific gravity of blood and 1.0086 as specific gravity of spinal fluid, the ratio between the specific gravities of these two fluids is 95.35.

CONCLUSIONS

In the cerebrospinal fluid in syphilis no constant deviation from normal is encountered in sugar, creatinin or urea content, in acid base equilibrium, in specific gravity, or in enzymatic activity.

⁶¹ Toison and Lenoble. Note sur la structure et sur la composition du liquide cephalorachidien chez l'homme. *Compt rend soc de biol* 43:373, 1891.

⁶² Hammersten. *Physiological Chemistry* 1914.

AN ANALYSIS OF NINETY CASES OF FUNCTIONAL DISEASE IN SOLDIERS *

JOHN M SWAN, M D

ROCHESTER, N Y

In "The Wrong Twin," by Harry Leon Wilson, there is a character, a civil war pensioner, who seems of particular interest at the present time Judge Penniman, whom Dave Cowan called "old Flapdoodle," had been granted a pension for his services in the Civil war The Judge had been obliged to abandon all thoughts of a career "He had been debarred by obscure ailments from active participation in our industrial strife" He could never perform the least work that required muscular effort, but he could always go to the harness shop for his game of checkers He complained of sciatica and of various other painful symptoms, "neuralgia mebbe" He always had the most comfortable chair available and was peevish if anyone else took it, he always demanded consideration and sympathy from his wife, whose earnings as a dressmaker, and his daughter, whose pay as a school teacher, served to piece out the income from the pension bureau He always took the newest patent medicines those containing the highest alcohol percentage preferred As "Old Doc Purdy" said "I had to think up some things that would get the old cuss his money, and dummed if he didn't take it all serious and think he did have 'em!"

It seems to me that the medical profession has an obligation to prevent, as far as may be, the development into chronic invalids of men discharged from the military service in the past two years and who are at present suffering from functional disease It is as a contribution to the understanding of this problem that the present paper is offered

MATERIAL

The study herewith presented is based on thirty-two cases which were referred to me for examination by the chief of the medical service or by the president of the disability board of Base Hospital 19 while it was operating in France, and fifty-eight cases referred to me between May 15 and December 31, 1920, in my capacity as group internist, U S Public Health Service in Rochester, New York, by the acting assistant surgeons, Dr Arthur P Reed and Dr Edgar W Phillips

In the former thirty-two cases there is only a record of the principal symptomatology and the findings on physical examination In the other fifty-eight cases more complete clinical investigations were made

* Read at the Thirty-eighth Annual Meeting of the American Climatological and Clinical Association, Lenox, Mass, June 3, 1921

Age—Five of the men were under 20 years of age, thirty-one were between 20 and 24, thirty-three between 25 and 29, seventeen between 30 and 35, three over 35, and the age of one was not recorded

Occupation—Table 1 gives the prewar occupations of fifty-eight patients Thirty-eight, 65.5 per cent, had been engaged in sedentary occupations, as follows Clothing industry one lining cutter Sub-sistence industries one waiter, one meat cutter, two cooks, one baker Manufacturing industries two machine operators, four instrument adjusters and assemblers of various kinds, one paper coater, one glass polisher, one automobile mechanic, one tire maker Printing industry one linotype operator, one lithographer Merchant class two managers, one merchant, one buyer Clerk class eight Students two Mis-

TABLE 1—OCCUPATIONS

No		No	
1035	Cook	1115	Camera assembler
1036	Lining cutter	1117	Farmer
1040	Laborer	1120	Clerk
1041	Machine operator	1121	Clerk
1042	Linotype operator	1123	Painter
1043	Farmer	1124	Machinist
1045	Stationary engineer	1128	Inspector in machine shop
1047	Rigger and dock builder	1129	Paper coater
1053	Buyer	1130	Clerk
1055	Night watchman	1131	Student
1058	Lithographer	1134	Elevator erector
1060	Cigar maker	1138	Clerk
1067	Painter	1142	Telephone assembler
1069	Automobile mechanic	1143	Manager of store
1073	Machinist	1144	Student
1078	Plumber	1146	Painter
1081	Retail clothing merchant	1148	Farmer
1082	Cook	1150	Tire maker
1085	Painter	1151	Laborer
1086	Advertising manager	1152	Draftsman
1088	Clerk	1156	Driver of delivery wagon
1094	Carpenter	1169	Shipping clerk
1096	Machinist	1170	Adjuster of scientific instruments
1097	Glass polisher	1171	Waiter
1099	Shipping clerk	1172	Farmer
1100	Telephone switchboard assembler	1173	Stock clerk
1112	Electrician	1174	Meat cutter
1113	Farmer	1175	Farmer
1114	Baker	1176	Gear cutting machine operator

cellaneous one driver of delivery wagon, one draftsman, one stationery engineer, one night watchman, one inspector, one cigar maker Twenty (34.4 per cent), had been engaged in nonsedentary occupations, as follows Farmers six Laborers two Rigger and dock builder one Building industries four painters, one carpenter, one plumber, one electrician, one elevator erector Manufacturing industries three machinists

Family History—The family histories of fifty-eight cases were studied Of these twenty-eight were negative Of the remaining thirty, 51.7 per cent, eighteen gave a history of nervous disease, four, of goiters, and six of both Of those who had a family history of nervous disease, there was one in which there was insanity, one in

which there was suicide, two cases of cerebrospinal syphilis, one case each of alcoholism, meningitis, melancholia and diabetes. Three families had deaths from carcinoma and two families had deaths from tuberculosis. In one case the patient's father and a brother had had gastro-intestinal attacks, and another brother had had convulsions in infancy.

Previous History—Table 2 gives the details of the previous histories of the ninety cases. In the analysis of these histories, only those diseases from which the patients had suffered since mobilization or those that had occurred within a year or two before mobilization are considered as having a possible etiologic significance on the condition for which the men applied for treatment.

In the first place, in only six is the previous history negative. All had been gassed, had had attacks of acute infectious disease, or had been wounded, with those exceptions. Twenty-four had had frequent attacks of tonsillitis, fourteen had had tonsillectomies, twenty had had frequent attacks of coryza or bronchitis, eight had had dysentery, nineteen had had influenza, twelve had had pneumonia, two had had boils, seven had had malaria, ten had had acute articular rheumatism, one had had gonorrheal rheumatism, three had had trench fever, one had had erysipelas, one had had septicemia, four had had catarrhal jaundice, five had had scarlet fever, one had had mumps, one had had cerebrospinal meningitis, one had had typhoid fever, four had had diphtheria, one had had rattlesnake bite, two had had goiters, one had had laryngitis, one had had diarrhea, two had had indefinite febrile attacks, two were tuberculosis suspects, three had had pleuritis, two had had otitis media, and seven had had operations: one gastroenterostomy, one mastoid operation, three appendectomies, one herniotomy, one had had six operations for the removal of foreign bodies in addition to shock and considerable hemorrhage at the time he was wounded.

Of the ninety men, fifty had been in action and forty had not been under fire. Of the fifty who had been in action, fifteen had received wounds, twenty-one had been gassed, and four had been both wounded and gassed.

In only ten cases was there a history of overuse of alcohol and of these two were questionable. In twenty-one cases there was a history of overuse of tobacco, one of which was questionable.

Chief Complaint—In fifty-eight cases, the chief complaint was recorded. Pain was the most frequent symptom for which the patients consulted a physician, twenty-two placing pain as their chief cause of distress. In eleven cases the pain was in the chest. Precordial pain, four, precordial pain with headache, one, precordial pain and lack of ambition, one, retrosternal pain, two, chest pain with cough and expect-

TABLE 2—PREVIOUS HISTORIES

Case No	
V 4	Fainted in action
V 5	Negative
V 8	Tonsillitis, gassed
V 9	Pneumonia, gunshot wound
V 17	Typhoid fever, scarlet fever, acute articular rheumatism, gassed
V 29	Pneumonia, gunshot wound
V 30	Gunshot wound
V 31	Diarrhea (2 attacks)
V 32	Gassed
V 33	Negative
V 34	Diphtheria, catarrhal jaundice, gunshot wound
V 40	Goiter, gunshot wound
V 45	Gassed and gunshot wound
V 47	Scarlet fever
V 49	Tonsillitis, diphtheria, gunshot wound
V 50	Pneumonia, acute articular rheumatism
V 54	Pneumonia, acute articular rheumatism, gunshot wound
V 55	Rattlesnake bite, indefinite fever, gunshot wound
V 57	Acute articular rheumatism, tonsillitis, scarlet fever
V 58	Tonsillitis, diphtheria, influenza
V 60	Malaria, tuberculosis
V 64	Tonsillitis, gunshot wound
V 67	Pneumonia, diphtheria, gunshot wound
V 69	Malaria
V 73	Malaria
V 81	Negative
V 82	Laryngitis
V 85	Bronchitis, indefinite fever
V 86	Gassed
V 93	Negative
V 94	Scarlet fever, influenza
V 95	Tonsillitis, acute articular rheumatism
1035	Influenza, tonsillectomy, gastro enterostomy
1036	Tonsillitis, tonsillectomy, influenza
1040	Gassed
1041	Gassed, gunshot wound
1042	Bronchitis, pneumonia, tuberculosis (suspect), pleurisy
1043	Dysentery, boils
1045	Malaria, tonsillitis, tonsillectomy, influenza
1047	Gonorrheal rheumatism, bronchitis, gassed
1053	Acute articular rheumatism, appendectomy, gassed
1055	Bronchitis
1058	Tonsillectomy, gunshot wound
1060	Tonsillectomy, otitis media
1067	Tonsillitis, tonsillectomy, influenza
1069	Dysentery, gunshot wound
1073	Tonsillitis
1078	Pleuritis, gassed
1081	Dysentery, acute articular rheumatism
1082	Acute articular rheumatism, influenza (2 attacks), mastoid operation
1085	Tonsillectomy with nasal operation, otitis media, gassed
1086	Influenza, pleuritis, coryza
1088	Bronchitis, trench fever, gassed
1094	Bronchitis
1096	Appendectomy, tonsillectomy, infected wound
1097	Bronchitis, influenza
1099	Dysentery, bronchitis, influenza
1100	Bronchitis, tonsillitis, tonsillectomy, influenza, gunshot wounds
1112	Shock and hemorrhage, 6 operations for foreign bodies, gunshot wounds
1113	Tonsillitis, tonsillectomy, mumps
1114	Tonsillitis, acute articular rheumatism, influenza, erysipelas, gassed
1115	Influenza
1117	Dysentery, tonsillitis, gassed, gunshot wound
1120	Pneumonia, influenza, malaria
1121	Pneumonia, malaria, tonsillitis, tonsillectomy, boils
1123	Trench fever, gassed
1124	Pneumonia, tonsillitis, septicemia
1128	Bronchitis
1129	Goiter
1130	Bronchitis, influenza, catarrhal jaundice
1131	Tonsillitis, nasal operation, catarrhal jaundice, gassed
1134	Appendectomy, gassed, gunshot wound
1138	Bronchitis, tonsillitis
1142	Scarlet fever, gassed
1143	Negative
1144	Dysentery, gassed
1146	Dysentery, malaria, acute articular rheumatism, bronchitis, gassed
1148	Dysentery, bronchitis, gassed
1150	Influenza, gunshot wound
1151	Bronchitis, tonsillitis, tonsillectomy, gassed
1152	Bronchitis, influenza, gassed
1156	Tonsillitis, gunshot wound
1169	Bronchitis, cerebrospinal meningitis
1170	Tonsillitis, tonsillectomy, melancholia, always sick and weak
1171	Tonsillitis, trench fever, gassed
1172	Influenza, pneumonia
1173	Pneumonia, bronchitis, gassed
1174	Pneumonia, bronchitis, influenza, catarrhal jaundice
1175	Negative
1176	Bronchitis, influenza, tonsillitis, pneumonia

toration, two, pain in lower border of left chest, one In four cases the pain was in the abdomen right lower abdominal quadrant, one, left upper abdomen, one, general abdominal pain with pyrosis, one, pain in "stomach" and knees, one In three cases the pain was in the back "soreness in side and back," one, pain in the back, one, pain in the back associated with nervousness, one Pain in the right shoulder and nervousness, one One patient complained of pain in the right wrist, one complained of pain in the neck and shoulders, and one complained of pain in both legs

Weakness was the next most frequent complaint, eleven patients complaining of this symptom Two complained of weakness only, one each of weakness and headache, weakness and dyspnea, weakness and sweating, weakness and pain in the chest, weakness and nervousness, lack of "pep," lack of "stamina," easily tired and "general run down condition" Nine patients complained of palpitation—four of palpitation alone and five of palpitation and dyspnea Four patients complained of "stomach trouble" "stomach trouble," one, "stomach trouble" and cold hands and feet, one, loss of appetite, one, indigestion, one Two complained of nervousness Three complained of dyspnea, one of them calling it "smothering and sinking spells" Of the remainder, one each complained of cough and expectoration, frequent micturition, headache, skin eruption and enlargement of the right posterior cervical lymph nodes One patient on discharge had been told that he had heart disease, and one came for examination for pension by one of the allied governments

Methods of Development—The following cases may be taken as typical of the development of the cases forming this group of functional diseases

REPORT OF CASES

CASE 1—A man, aged 23, was gassed July 11, 1918 He was admitted to Base Hospital 19 four days later He had some cough and a few râles over the larger bronchi, but was apparently doing well until five weeks later, when he had a sudden pain in his left side, accompanied by surface coldness and sweating Since then he had complained of paroxysmal dull pain in the chest, which radiated down the right side of the sternum and across the base of the chest It did not radiate to the neck or the arms This pain frightened him He also complained of dyspnea, palpitation and arrhythmia

CASE 2—A man, aged 20, was admitted to Base Hospital 19 Aug 7, 1918, for gunshot wound of the hand He had enlisted in the National Guard, State of Michigan, in 1917, and in October was transferred to the medical department from the infantry on account of goiter After about four months treatment, the goiter became smaller, and he was transferred back to the infantry In April, 1918, he noticed that his goiter was beginning to return, and he began to complain of blurring of vision, which interfered with his marksmanship, and black specks before his eyes He also complained of frontal headache, thumping in the back of his head, dyspnea and palpitation of the heart on exertion

CASE 3—A man, aged 26, was admitted to Base Hospital 19 July 31, 1918, for gunshot wound of the calf of the left leg and exposure to phosgene gas. He complained of sharp paroxysmal precordial pain, always once a day, sometimes more frequently. He also complained of pain in the left side of the neck and in the left arm, dyspnea and palpitation of the heart on exertion, and spontaneous and nocturnal dyspnea with subjective arrhythmia.

CASE 4—A man, aged 25, was admitted to Base Hospital 19, Sept. 25, 1918, for a wound in the right chest incurred in action September 12. While in Base Hospital 19 he did well, the wound healed without operative procedure. When he was first allowed out he noticed dyspnea on exertion and palpitation of the heart. These symptoms increased and were later attended with shooting pains in the chest, slight cough, with the expectoration of a yellowish mucus without blood, occasional nausea and nervousness.

CASE 5—A man, aged 24, was admitted to Base Hospital 19 for laryngitis. From the time of his induction into the service he had been in hospitals most of the time. The laryngitis was thought by Major Edward L. Hanes, the neurologist and president of the disability board, to be hysterical. He complained of indefinite chest pain, occasional cough, expectoration and arrhythmia.

CASE 6—A man, aged 29 years, who claimed to have been perfectly well before he was inducted into the service, began to complain of attacks of trembling, headache, palpitation and dyspnea as the result of hikes in training camp and following the second dose of antityphoid vaccine. The first attack came on during the night, wakening him, and lasted about fifteen minutes. He felt tired and nervous after it and did not go to sleep again that night. The next day he went out to drill, and while doing double time, he became dizzy and fell down. He was put on light duty, but had similar attacks two or three times a week. On his return to full duty, he had the attacks at night and sometimes in the daytime. During the voyage to France, and for the first week after landing, he felt pretty well, but as soon as training began, the attacks returned with increasing severity. He denied that fear played any part in the production of the attacks. He was returned to the United States as a sick casual. The attacks continued after his return to the United States and persisted for thirteen months after his discharge.

CASE 7—A man, aged 24, who had been in two major offensives and in the third Army, and had been wounded, noticed that he was nervous about six weeks after his discharge. His heart beat too fast, he would get out of breath when he was hurried or excited, and his hands trembled. He also complained of being weak and tired, and of loss of weight. These symptoms had been present for a year at the time of his first examination.

CASE 8—A man, aged 27, was assigned to the horseshoer's school at one of the camps. In the latter part of 1918, he began to complain of frequent micturition, passing urine from eight to sixteen times a day and from one to three times at night. About two weeks later, he began to wet the bed and to dribble in the daytime. The symptoms persisted twenty months after his discharge. While discussing his symptoms he began to cry. He said that he had lost two jobs since his discharge from the army, a thing that had never happened before. He said that at the horseshoer's school he had to work with mules that had never been shod before, and that sometimes it was necessary to have twelve men hold an animal while he was being shod. Every day men were knocked out while at this work and some men were killed. This work got on his nerves and he became afraid of the animals.

CASE 9—A man, aged 27, answered sick call a good deal while in the service. His feet hurt him and he could not do much guard duty or drill. About a month after his discharge he began to complain of attacks characterized by a rising in his chest which came up to the top of his breast bone and choked him, making him short of breath and "awful" nervous. The attacks came on about one-half hour after eating. He had vomited but once—during the first attack.

CASE 10—A man, aged 29, was gassed in action. He recovered and returned to duty. About five months later, he was hospitalized with general edema, delirium and suppression of urine, and a diagnosis of nephritis was made. Eighteen months after his discharge he was complaining of pain in his back, nervousness, vertigo, tinnitus, black specks and flashes of light, nasal discharge, bad taste in his mouth, cough with the expectoration of yellowish mucus, loss of weight, dyspnea and palpitation on exertion and excitement, abdominal cramps with pyrosis and nausea, gas in his stomach, loss of memory, anxiety and ugly temper.

CASE 11—A man, aged 25, had pneumonia three weeks after reporting at the training camp. After recovery he was assigned to an infantry company and found that he could not carry his pack or do long hikes. He began to complain of palpitation, dyspnea and dull precordial pain as soon as he was out of bed. Two years after his discharge he was still complaining.

SYMPTOMATOLOGY

The symptoms brought out by questioning were: loss of consciousness, twelve cases; headache, forty-seven cases; tinnitus, twenty-one cases; vertigo, fifty cases; chest pain, fifty-one cases; dyspnea, sixty-seven cases; palpitation, sixty-six cases; subjective arrhythmia, twenty-two cases; cough, fifty cases; expectoration, thirty-nine cases; abdominal pains, twenty-one cases; nausea, twenty-one cases; vomiting, eleven cases; sweating, thirty-one cases; nervousness, fifty cases; anorexia, twenty-seven cases; insomnia, thirty-eight cases; constipation, fourteen cases; diarrhea, ten cases; alternating constipation and diarrhea, four cases; sensation of shaking, one case; amblyopia, five cases.

An analysis of the cases that presented chest pain showed that it was precordial in thirty-three, retrosternal in eight, in the right chest in two, below the right scapula in one, indefinite in three, and in one each it was described as "soreness," "a depressed feeling in the chest," a sensation of "squeezing," and in the lower chest. Two patients complained of pain in the neck.

Fifty of fifty-eight patients were not in the habit of bathing with sufficient frequency, and thirty-seven habitually took little or no exercise.

Physical Signs—The physical signs may be divided into three groups. First, a group of general nutritional and developmental disturbances, second, a group indicating disturbance of the endocrine system, and third, a cardiovascular group.

Nutritional and Developmental Disturbances—Of the fifty-eight cases in which this was studied, forty-five, or 77.5 per cent, were underweight, thirty-eight less than twenty-five pounds, seven more than twenty-five pounds, maximum, thirty-nine pounds, minimum, one pound. Six were normal in weight for their age and height. Seven were overweight. Maximum, twenty-one pounds, minimum, two pounds.

Table 3 shows the frequency of enlargement of the superficial lymph nodes. The enlargement was always small, amounting to an ability to palpate the glands. The posterior cervical and inguinal enlargements may be disregarded as this enlargement is almost universal. The axillary lymph nodes were palpable in twenty-four cases, the epitroch-

TABLE 3—PALPABLE SUPERFICIAL LYMPH NODES

Case No	Nodes Enlarged
1035	Posterior cervical, axillary, inguinal
1036	Posterior cervical
1040	Epitrochlear, inguinal
1041	Posterior cervical
1042	Posterior cervical, right axillary, right epitrochlear, inguinal
1043	Posterior cervical, left epitrochlear, inguinal
1045	Posterior cervical, axillary, inguinal
1047	Posterior cervical, axillary, inguinal
1053	None
1055	Posterior cervical, axillary, inguinal
1058	Posterior cervical, axillary, inguinal
1060	None
1067	Posterior cervical, axillary, inguinal, epitrochlears questionable
1069	Posterior cervical
1073	Posterior cervical, right axillary, inguinal
1078	Posterior cervical, axillary, inguinal
1081	Posterior cervical, inguinal
1082	Posterior cervical, inguinal
1085	Posterior cervical, inguinal
1086	Right posterior cervical, right anterior cervical, inguinal
1088	Posterior cervical, inguinal
1094	Posterior cervical, axillary, epitrochlear, inguinal
1096	Inguinal
1097	Posterior cervical, axillary, left epitrochlear, inguinal
1099	Posterior cervical, inguinal
1100	Posterior cervical, inguinal
1112	Posterior cervical, left supraclavicular, axillary, epitrochlear, inguinal
1114	Posterior cervical, left supraclavicular, epitrochlear, inguinal
1115	Posterior cervical, supraclavicular, epitrochlear, inguinal
1117	None
1120	Posterior cervical, epitrochlear, inguinal
1121	None
1128	Posterior cervical, supraclavicular, epitrochlear, inguinal
1129	None
1130	Posterior cervical, axillary
1131	Posterior cervical, supraclavicular, left axillary, epitrochlear, inguinal
1134	Posterior cervical, inguinal
1138	Posterior cervical, axillary, right epitrochlear, inguinal
1142	Posterior cervical, inguinal
1143	Posterior cervical, axillary, inguinal
1144	Posterior cervical, axillary, left epitrochlear, inguinal
1146	Posterior cervical
1148	Posterior cervical, supraclavicular, axillary, epitrochlear, inguinal
1150	Posterior cervical, supraclavicular, epitrochlear, inguinal
1151	Posterior cervical, epitrochlear, inguinal
1152	Posterior cervical, supraclavicular, inguinal
1156	Posterior cervical, axillary, inguinal
1169	Posterior cervical, inguinal
1170	Posterior cervical, epitrochlear, inguinal
1171	Posterior cervical, inguinal
1172	Posterior cervical, inguinal
1173	Posterior cervical, axillary, left epitrochlear, inguinal
1174	Posterior cervical, axillary, epitrochlear, inguinal
1175	Posterior cervical, axillary, epitrochlear, inguinal
1176	Posterior cervical, axillary, epitrochlear, inguinal

lears in twenty-three, the anterior cervicals in one case, and the supraclaviculars in eight cases. There were no palpable superficial lymph nodes in five cases. Among the other signs in this class were noted High palatine arch, three cases, scoliosis, one case, anteroposterior spinal curve, one case, hypertrichosis, two cases, hypospadias, one case, short stubby fingers, one case, spaced teeth, three cases, phimosis,

one case One patient was a stutterer and one presented moderately marked gastrectasia

Endocrine Group—Lost occipitofrontalis control, twenty-nine cases, Dalrymple's sign, twenty-nine cases, nystagmus, twenty-five cases, Mobius' sign, fourteen cases, von Graefe's sign, six cases, the ocular movements were irregular in fifteen cases, blepharospasm, four cases, lacrimation, four cases, too frequent winking, six cases, exaggerated reaction to light, one case The ocular movements were normal in thirty-one cases, or 34.4 per cent Pigmented eyelids were noted in two cases Tremors were present in varying combination In the eyelids, in fifty cases, in the tongue, in thirty-five cases, in the hands, in thirty-three cases, in the lips in eleven cases There was a general muscular tremor in five cases There was a tremor of the fibers of the occipitofrontalis muscle in two cases, and athetoid movements of the tongue were noted in two cases Twenty-one patients, 23.3 per cent showed no tremors Dermographia was present in varying intensity in fifty-five cases, goiter was present in forty-five cases, or 50.0 per cent, of these the goiter was large in five cases The thyroid body was palpable with difficulty in five cases Exophthalmus was present in two cases

Cardiovascular Group—The oblique diameter of cardiac dulness was under 15 cm in seventy-eight cases, in twelve it was over 15 cm, maximum, 17.5 cm Murmurs were present in nineteen cases "Systolic murmur heard all over the precordium in the standing posture, not heard in the recumbent posture," three cases "Soft systolic murmur all over the body of the heart," five cases "Systolic murmur at the apex transmitted into the axilla, in the second left interspace, and over the body of the heart," one case "Soft systolic murmur in the second left interspace," eight cases "Soft systolic murmur at the apex in the standing posture, disappears in the recumbent posture," two cases The aortic diastolic sound was louder than the pulmonary diastolic sound in thirty-two cases, the pulmonary diastolic sound was louder than the aortic diastolic sound in twenty cases, and the two sounds were equal in thirty-three cases No note was made in five cases Extrasystoles were present in five cases and a sinus arrhythmia in four cases

Pulse Table 4 gives the pulse rates of these patients in the recumbent posture, the standing posture, after exercise and after two minutes rest In the V series of cases exercise consisted of mounting a flight of twenty-four steps, each step 6½ inches high, equivalent to lifting the patient's body thirteen feet In the other cases, exercise consisted of twenty-five stationary hops on each foot In the recumbent posture thirty-one patients had a pulse rate of over eighty, twenty-nine had a pulse rate of under seventy, and thirty had a pulse rate of

TABLE 4—PULSE RATES

No	Recumbent	Standing	After Exercise	After Two Minutes	Increase on Standing	Increase After Exercise	Per Cent of Increase on Standing
V 4	70	92	116		22	46	47 8
V 5	78	90	98		12	20	60 0
V 8	84	96	112		12	28	42 8
V 9	106	106	116		0	10	0 0
V 17	82	110			28		
V 29	108	118	128		10	20	50 0
V 30	80	116	122		36	42	85 7
V 31	72	92	108		20	36	55 5
V 32	88	90			2		
V 33	106	118			12		
V 34	86	96			10		
V 40	70	102			32		
V 45	92	94			2		
V 47	80	90	122		10	42	23 8
V 49	100	112	120		12	20	60 0
V 50	76	98			22		
V 54	76	90			14		
V 55	82	112			30		
V 57	66	86			20		
V 58	80	118			38		
V 60	134						
V 64	50						
V 67	110	140			30		
V 69	122	122			0		
V 73	92	106	120		14	28	50 0
V 81	112	120			8		
V 82	82	100			18		
V 85	90	110			20		
V 86	96	116			20		
V 93	72	80			8		
V 94	94	94			0		
V 95	84	80			-4		
1035	66	68			2		
1036	70	88			18		
1040	74	96			22		
1041	70	96			26		
1042	64	96			32		
1043	94		118	98		24	
1045a	60	66	86	60	6	26	23 0
1047	76	92	98	74	16	22	77 7
1053	74	102	110	102	28	36	77 7
1055	66	74			8		
1058	82	94	98	84	12	16	75 0
1060	84	100	100	88	16	16	100 0
1067	70	98	118	80	28	48	58 3
1069	68	94			26		
1073	66	80			14		
1078	50	70	82	68	20	32	62 5
1081	54	58			4		
1082	54	74	90	58	20	36	55 5
1085	82	84			2		
1086	62	68			6		
1088	70	94	100	74	24	30	80 0
1094	62	80			18		
1096	68	74	94	56	6	26	23 0
1097	78	88	96	80	10	18	55 5
1099	68	88	94	68	20	26	76 9
1100	60	86	80	80	26	20	130 0
1112	80	98	100	86	18	20	90 0
1113	62	64	74	64	2	12	16 6
1114	78	92	90	72	14	12	116 6
1115	94	110	114	78	16	20	80 0
1117	80	106	118	94	26	38	68 4
1120	78	96	98	82	18	20	90 0
1121	92	94	106	108	2	14	14 2
1123	72	78	96	74	6	24	25 0
1124	66	82	86	62	16	20	80 0
1128	54	62	84	66	8	30	26 6
1129	90	112	112	94	22	22	100 0
1130	74	94	110	96	20	36	55 5
1131	64	88	90	72	24	26	92 3
1134	64	94	90	72	30	26	115 3
1138	70	94	88	70	24	18	133 3
1142	80	94	94	80	14	14	100 0
1143	78	106	96	74	28	18	155 5
1144	74	82	84	74	8	10	80 0
1146	62	78	80	62	16	18	88 8
1148	86	94	104	94	8	18	44 4
1150	96	116	104	90	20	8	250 0
1151	66	80			14		
1152	66	72	84	60	6	18	33 3
1156	82	86	96	78	4	14	28 5
1169	68	76	84	56	8	16	50 0
1170	92	98	98	84	6	6	100 0
1171	66	78	78	58	12	12	100 0
1172	66	72	88	60	6	22	27 2
1173	74	84	94	72	10	20	50 0
1174	76	98	98	80	22	22	100 0
1175	62	86			24		
1176	64	84			20		

between seventy and eighty, inclusive. This table shows the increase per minute in the standing posture and after exercise (pulse counted $\frac{1}{2}$ minute), and gives the percentage of increase produced by the change from the recumbent to the standing posture. I have analyzed the increase in the pulse rate on standing in another communication¹. In the former series, the increase on standing was in excess of twelve beats (taken as normal) in thirty-nine out of ninety-six observations, or 40.6 per cent. In this series, the increase on standing was above twelve beats in forty-nine out of sixty-seven observations, or 73.1 per cent. In fifty-two cases in which the increase on standing and after exercise is recorded, that produced by changing the position formed more than 50 per cent of the increase in thirty-five cases, or 67.3 per cent. And in twelve cases, or 23 per cent, the increase on standing was more than the increase after exercise, in one case it amounted to 250 per cent of the increase after exercise. These figures seem to me to indicate the nervous character of the changes in the pulse rate in these cases. I am led to conclude that as much evidence can be obtained as to the irritability of the pulse by observing the increase on standing as by noting the increase after exercise.

In eight cases, the pulse failed to return to within ten beats of the original rate in the recumbent posture within two minutes after exercise. I am of the opinion that the failure of the pulse to return to the original rate within two minutes cannot be taken as a positive indication of heart muscle weakness. In thirteen cases the pulse rate two minutes after exercise was below the rate in the recumbent posture before exercise was taken.

Blood Pressure. Table 5 shows the systolic, diastolic and pulse pressures in eighty-nine of the cases in the recumbent posture. The pressures in the V series were taken with the Tycos instrument, the remainder were done with a Riva Rocci instrument. In reading the diastolic pressure, I have used the fifth point, as indicated in a former communication². In general the V cases showed higher systolic and diastolic pressures than the cases seen two years later. The systolic pressure was below 120 mm in sixteen cases, between 120 and 130 mm in thirty-six cases, and over 130 mm in thirty-seven cases. The maximum systolic pressure observed was 160 mm in two cases. The diastolic pressure was below 40 mm in one case, between 40 and 50 mm in two cases, and over 50 mm in eighty-six cases. Maximum, 100 mm in one case. The pulse pressure was between 20 and 30 mm in two cases, between 31 and 40 mm in nine, between 41 and 50 mm in twenty, between 51 and 60 mm in twenty-eight, between 61 and 70

1 Swan Internat Clin 2 3 113, 1916

2 Swan Internat Clin 4 1, 1914

mm in nineteen, between 71 and 80 mm in eight, between 81 and 90 mm in two cases, and 110 mm in one case

Laboratory Examinations—Urine Routine urine examinations were made in fifty-eight cases, seventy-six examinations being made In eighteen, the specific gravity was normal (between 1 015 and 1 020) In twenty-three it was low, lowest, 1 006 In thirty-five it was high, highest 1 035 Albumin was present in small quantity in four cases Glucose was present in six cases, three of which were questionable

TABLE 5—BLOOD PRESSURES

Case No	Systolic	Diastolic	Pulse Pressure	Case No	Systolic	Diastolic	Pulse Pressure
V 4	128	70	58	1073	140	80	60
V 5	138	54	64	1078	106	70	36
V 8	124	62	62	1081	120	80	40
V 9	138	80	58	1082	142	78	64
V 17	124	74	50	1085	114	64	50
V 29	140	86	54	1086	118	80	38
V 30	140	70	70	1088	130	78	52
V 31	136	70	66	1094	120	68	52
V 32	138	88	50	1096	120	85	35
V 34	136	78	58	1097	140	62	78
V 40	130	66	64	1099	115	70	45
V 45	140	84	56	1100	115	68	47
V 47	148	88	60	1112	135	80	55
V 49	160	80	80	1113	160	90	70
V 50	138	70	68	1114	118	78	40
V 54	158	70	88	1115	140	70	70
V 55	122	76	46	1117	127	75	52
V 57	134	70	64	1120	128	80	48
V 58	138	64	74	1121	148	80	68
V 60	150	40	110	1123	135	68	57
V 67	144	100	44	1124	128	85	43
V 64	106	52	54	1128	119	68	51
V 69	150	86	64	1129	136	50	86
V 73	136	60	76	1130	120	65	55
V 81	128	90	38	1131	105	30	75
V 82	124	70	54	1134	112	70	42
V 85	104	80	24	1138	130	75	55
V 86	140	86	54	1142	124	65	59
V 93	150	80	70	1143	123	65	58
V 94	158	96	62	1144	122	75	47
V 95	146	76	70	1146	122	74	48
1035	100	70	30	1148	136	65	71
1036	120	80	40	1150	120	80	40
1040	147	80	67	1151	122	80	42
1041	130	65	65	1152	122	65	57
1042	129	55	74	1156	120	65	55
1043	130	80	50	1169	129	75	54
1045a	107	70	37	1170	137	95	42
1047	127	78	49	1171	116	70	46
1053	115	64	51	1172	132	80	52
1055	125	54	71	1173	128	75	53
1058	130	70	60	1174	112	60	52
1060	124	79	45	1175	146	80	66
1067	125	80	45	1176	126	60	66
1069	133	89	44				

Phosphates were present in forty-one cases and carbonates in twenty cases In fifteen cases calcium oxalate crystals were found in the sediment, in thirteen cases, pus, mucus and epithelium were present, in nine cases uric acid crystals were found Casts were seen in four cases, renal epithelium in one case, and red blood corpuscles in one case

Blood Table 6 gives the results of the blood counts in fifty-eight cases The erythrocytes were over five million in all except four cases maximum, 6,320,000, minimum, 4,740,000 The hemoglobin per-

centage (Sahl instrument) was below 90 in eleven cases, between 90 and 100 in twenty-nine cases, over 100 in eighteen cases maximum, 107 per cent, minimum, 83 per cent The color index, on the other

TABLE 6—BLOOD COUNTS

Case No	Erythrocytes	Leukocytes	Hemoglobin	Color Index	Polymorphonuclear Neutrophils		Lymphocytes	
					Absolute	Per Cent	Absolute	Per Cent
1035	5,010,000	6,320	90	0.89	4,183	66.2	1,971	31.2
1036	5,060,000	4,880	98	0.96	3,347	68.6	1,327	27.2
1040	5,190,000	10,720	89	0.85	8,468	79.0	2,122	19.8
1041	5,430,000	5,040	92	0.84	3,407	67.6	1,612	32.0
1042	5,030,000	5,680	93	0.92	3,532	62.2	1,885	33.2
1043	5,650,000	7,600	83	0.73	4,864	64.0	2,599	34.2
1045a	5,990,000	6,480	94	0.77	4,017	62.0	2,099	32.4
1047	5,730,000	10,000	87	0.76	5,440	54.4	4,100	41.0
1053	5,690,000	9,360	91	0.79	5,709	61.0	3,444	36.8
1055	5,390,000	7,040	94	0.87	3,674	52.2	3,027	43.0
1058	5,300,000	9,680	93	0.87	6,369	65.8	2,884	29.8
1060	5,900,000	6,960	99	0.83	5,136	73.8	1,642	23.6
1067	5,860,000	8,000	105	0.89	4,986	61.2	2,928	36.6
1069	5,280,000	12,480	104	0.98	8,062	64.6	4,943	32.4
1073	5,250,000	12,000	95	0.90	7,968	66.4	3,760	31.0
1078	5,610,000	9,120	92	0.81	5,782	63.4	2,772	30.4
1081	5,700,000	9,440	92	0.80	5,526	59.6	3,398	36.0
1082	5,260,000	10,000	102	0.96	5,980	59.8	3,180	31.8
1085	5,830,000	6,480	98	0.84	4,199	64.8	1,866	28.8
1086	5,010,000	6,640	87	0.86	3,373	50.8	2,589	39.0
1088	5,850,000	8,320	104	0.88	4,792	57.6	3,111	37.4
1094	5,760,000	13,040	86	0.75	10,066	77.2	2,686	20.6
1096	5,300,000	7,680	97	0.91	4,577	59.6	2,488	32.4
1097	5,250,000	7,440	101	0.96	5,034	67.8	1,993	26.8
1099	6,120,000	10,800	110	0.89	6,827	62.8	3,724	32.4
1100	5,450,000	6,880	92	0.84	4,004	58.2	2,655	38.6
1112	4,840,000	12,160	107	1.10	7,928	65.2	3,193	31.2
1113	5,370,000	9,600	94	0.87	7,372	76.8	1,920	20.0
1114	5,100,000	16,880	95	0.93	13,976	82.8	2,160	12.8
1115	5,670,000	9,120	95	0.83	7,277	79.8	1,550	17.0
1117	6,320,000	10,480	102	0.80	7,021	67.0	2,766	26.4
1120	5,290,000	16,720	105	0.99	11,169	66.8	4,781	28.6
1121	6,210,000	7,440	96	0.77	4,330	58.2	2,916	39.2
1123	5,170,000	9,520	97	0.93	5,064	53.2	4,188	44.0
1124	5,240,000	8,160	100	0.95	4,977	61.0	2,709	33.2
1128	5,880,000	12,880	99	0.84	8,011	62.2	4,173	32.4
1129	5,480,000	15,200	110	1.00	11,521	75.8	3,070	20.2
1130	5,710,000	10,560	106	0.92	7,455	70.6	2,534	23.4
1131	6,180,000	8,480	90	0.72	5,698	67.2	2,204	26.0
1134	5,500,000	7,280	99	0.90	3,552	48.8	3,221	44.8
1138	5,690,000	11,920	102	0.89	4,934	41.4	5,745	48.2
1142	5,020,000	8,320	85	0.84	4,609	55.4	2,745	33.0
1143	5,160,000	8,640	84	0.81	4,907	56.8	2,972	34.4
1144	5,570,000	7,440	89	0.79	3,675	49.4	2,990	40.2
1146	4,740,000	5,280	106	1.11	3,009	57.0	1,985	37.0
1148	4,910,000	11,920	87	0.88	7,438	62.4	3,576	30.0
1150	6,310,000	9,440	104	0.82	6,041	64.0	2,888	30.6
1151	5,350,000	11,520	105	0.98	7,787	67.6	3,133	27.2
1152	5,530,000	7,040	94	0.84	3,632	51.6	2,956	42.0
1156	5,120,000	11,920	88	0.85	7,533	63.2	4,124	34.6
1169	5,120,000	10,160	89	0.86	7,660	75.4	2,052	20.2
1170	5,310,000	8,000	102	0.96	5,456	68.2	1,984	24.8
1171	5,480,000	11,760	105	0.95	5,456	60.0	1,984	32.4
1172	4,800,000	9,920	101	1.05	6,784	68.4	2,519	25.4
1173	5,650,000	11,600	96	0.84	10,744	84.0	1,322	11.4
1174	4,940,000	14,160	97	0.98	11,752	83.0	1,755	12.4
1175	4,970,000	7,440	97	0.97	4,925	66.2	2,172	29.2
1176	6,090,000	9,840	91	0.74	7,202	73.2	2,164	22.0

hand, was below 0.90 in thirty-six cases, or 62.0 per cent, from 0.70 to 0.79 in nine cases, from 0.80 to 0.89 in twenty-seven cases, from 0.90 to 0.99 in eighteen cases, above 1.0 in four cases maximum, 1.11, minimum, 0.72 The leukocyte count was normal (between 8,000

and 10,000) in nineteen cases, below 8,000 in nineteen cases and over 10,000 in twenty cases, normal, 32.7 per cent, leukopenia, 32.7 per cent, leukocytosis, 34.6 per cent maximum, 16,880; minimum, 4,880. The polymorphonuclear neutrophil leukocytes were normal (from 4,960 to 7,000 per c mm) in twenty-two cases. There was a polymorphonuclear, leukocytosis (above 7,000 per c mm) in twenty cases, and a polymorphonuclear leukopenia (below 4,960 per c mm) in sixteen cases normal, 37.9 per cent, leukocytosis, 34.4 per cent, leukopenia, 27.5 per cent highest, 13,976, lowest, 3,009. The lymphocytes were normal (from 1,600 to 3,000 per c mm) in forty cases. There was a lymphocytosis in fifteen cases and a lymphopenia in three cases, lymphocytosis in 25.8 per cent highest, 4,943, lowest, 1,322. High eosinophil percentages were obtained in three cases 4, 4.2 and 5 per cent, respectively. My results parallel those of Levy.³ Wassermann tests were done in eleven cases, all were negative. Five sputum examinations were made, in none were acid fast bacilli found. Blood chemistry examinations were made in four cases, in none was nitrogen retention or hyperglycemia found.

Complications—Table 7 shows the complicating conditions recorded in sixty-nine of the ninety cases. In twenty-two cases, dental infection was found, in thirteen cases mouth infection, in two pyorrhea. Tonsillitis was present in twenty-three cases, nasopharyngitis in eleven cases, furunculosis in two cases, pediculosis in one case, nose bleed in one case, hemorrhoids in five cases, acute bronchitis in two cases, prostatitis in two cases, acute rhinitis in one case; gonorrhea in one case, psoriasis in one case, and multiple arthritis in one case. In nine cases, or 13.0 per cent, no complicating conditions were found.

CLINICAL DIAGNOSIS

What name shall we give these cases? A diagnosis had to be made at the time of the first examination of the patients and my endeavor was to classify them as belonging to the group indicated by the most prominent clinical feature. On that basis a diagnosis of effort syndrome was made in twenty-eight cases, of hyperthyroidism in five cases, of dysthyroidism in twenty-four cases, of chloroanemia in eleven cases, of traumatic neurosis in five cases, of goiter in three cases, of dyspituitarism in two cases, of mouth infection in two cases, of neurasthenia in two cases, and of ductless glandular disease, paroxysmal tachycardia, extrasystolic arrhythmia, myxedema, pyorrhea alveolaris, peritoneal adhesions, pulmonary emphysema, and nervous dyspepsia in one case each.

³ Levy Brit M J 2 715, 1917

The cases were named irritable heart of soldiers by DaCosta in 1871, effort syndrome by Lewis and his co-workers⁴ in 1917, disordered heart action (D A H) and valvular disease of the heart (V D H) in the nomenclature of the medical department of the British Army. They have also been called neurasthenia (vasomotor type), heart strain, irritable heart, soldier's heart, athlete's heart, cardiovascular asthenia and neurocirculatory asthenia.

We have here a group of cases occurring in men who have been in the military service, recruited from occupations of a sedentary

TABLE VII—COMPLICATIONS

V 31	Chronic tonsillitis	1097	Mouth infection, chronic tonsillitis
V 45	Mouth infection, diffuse bronchitis	1099	Chronic pharyngitis, acute bronchitis
V 50	Mouth infection, multiple arthritis	1100	None
V 54	Tonsillitis, chronic	1112	Chronic tonsillitis
V 57	Chronic tonsillitis	1113	Mouth infection
V 69	Mouth infection	1114	Mouth infection
V 73	Mouth infection	1115	None
V 81	Mouth infection, chronic tonsillitis	1117	Mouth infection
V 85	Mouth infection, chronic tonsillitis	1120	Mouth infection
V 86	Mouth infection	1121	None
1035	Mouth infection	1123	None
1036	Pyorrhea alveolaris, furunculosis	1124	Chronic tonsillitis
1040	Pediculosis pubis and corporis	1128	Chronic tonsillitis, gastritis
1041	Chronic tonsillitis, nasopharyngitis, furunculosis	1129	Chronic pharyngitis
1042	Chronic pharyngitis, nose bleed	1130	Chronic tonsillitis
1043	Mouth infection	1131	None
1045a	Mouth infection	1134	Pulmonary emphysema
1047	Hemorrhoids	1138	Mouth infection, chronic pharyngitis
1053	Mouth infection, chronic tonsillitis and pharyngitis	1142	None
1055	Chronic tonsillitis	1143	Mouth infection
1058	Mouth infection, chronic pharyngitis	1144	Chronic tonsillitis
1060	Mouth infection, dilated and ptosed stomach	1146	Mouth infection, hemorrhoids, prostatitis
1067	None	1148	Mouth infection, prostatitis
1069	Mouth infection	1150	Mouth infection, chronic tonsillitis
1073	Chronic tonsillitis and pharyngitis	1151	Mouth infection, acute rhinitis
1078	None	1152	Chronic tonsillitis
1081	Mouth infection	1156	Mouth infection, chronic tonsillitis
1082	Mouth infection and chronic pharyngitis	1169	Hemorrhoids
1085	Mouth infection	1170	Hemorrhoids
1086	Mouth infection, chronic tonsillitis	1171	Mouth infection, acute urethritis
1088	Chronic tonsillitis	1172	Chronic tonsillitis
1094	Mouth infection, chronic pharyngitis	1173	Hemorrhoids
1096	Mouth infection, cholecystitis (suspected)	1174	Chronic tonsillitis and pharyngitis, mouth infection
		1175	Mouth infection
		1176	Mouth infection, psoriasis

type in 65.5 per cent, and with a family history of nervous disease in 51.7 per cent, who, as a result of wounds, gassing, acute infections, or the stress and strain of military life have broken down. They present a symptomatology which in its groupings is of the functional type, on physical examination, they present no evidence of disease of an organic nature, or if they do the evidence is found on closer study to be transitory. The cases are associated with a history of previous subacute or chronic infections in 89.6 per cent of the patients and they

⁴ Lewis. Report upon Soldiers Returned as Cases of "Disordered Action of the Heart" (D A H) or "Valvular Disease of the Heart" (V D H). London, 1917.

present complications of an infective character (tonsillitis, nasopharyngitis, sinusitis, infected teeth) in 87 per cent. In about 50 per cent of the cases, there are physical signs which point to a disturbance of the endocrine system, with small goiter. In all cases there is some evidence of disturbance of the ductless gland system. Is the infection the cause of the condition or is the endocrine disturbance the cause of it? Lewis and his co-workers are of the opinion that the infection, by setting up faulty metabolism, is the cause of the disturbance, the abnormal metabolic products producing the symptoms. If this be so, it would seem that the removal of the focus of infection should be followed by relief of the symptoms. This is not so. Fourteen of the fifty-eight patients seen after discharge from the service had had tonsillectomies, and their symptoms were still present, albeit relieved in some degree. It seems to me that the blood counts ought to be of importance in deciding the question. If the infection were the chief factor in the symptom complex, I think we ought to see a polymorphonuclear leukocytosis more frequently than in 34.4 per cent. As a matter of fact, the polymorphonuclear leukocytes are frequently reduced, 27.5 per cent. I am at the present time strongly inclined to the opinion that the endocrine disturbance is the responsible factor, responsible for the infection and responsible for the breakdown which these men experience. If the polymorphonuclear neutrophil leukocytes carry the antibodies that combat infection, certainly a blood containing only 3,000 of these cells per c. mm. is deficient in protective substances. Sewall⁵ is of the opinion that many of these cases are caused by "occult tuberculosis." In examining these men, tuberculosis has been constantly in mind. In one case, a diagnosis of tuberculosis was made in a hospital, but I could not agree that the physical signs presented by the patient warranted such an interpretation. In twenty-three out of fifty-eight cases, 39.6 per cent, prolonged expiration at the right apex was noted, but no signs that in my judgment would warrant an interpretation of tuberculosis were present.

TREATMENT

The problem in the treatment of these cases is to convert a man who is on the road to becoming an introspective, neurasthenic and hypochondriacal invalid into a wholly or partially self supporting and self respecting member of the community. The treatment is very largely psychic. In the first place, the man should have a careful and thorough modern clinical study of his case made. He must be allowed to tell his own story and bring out his own attitude toward the cause of his symptoms and their development. He should then be

⁵ Sewall. *Am J M Sc* **158** 786, 1919

questioned as to the existence of symptoms of which he may have said nothing. Then, a thorough routine physical examination, with a urine examination and a blood count, should be made. When indicated, he should be referred to the ophthalmologist, the otolaryngologist, the roentgenologist, the neurologist, the orthopedist, the surgeon, and he should have blood chemistry studies and a Wassermann examination made. All existing local or focal infections should be removed. Then, he should be taught the principles of personal hygiene, regular habits, diet, care of the mouth, reduction of use or elimination of tea, coffee, alcohol and tobacco, the correction of constipation, daily bathing, sleeping with windows open, exercise. The insistence on the latter, under proper supervision, is one of the most important features of the routine. Then he should have erroneous ideas of cause and effect in regard to his complaints explained away. After that he should be encouraged to go to work and stay at work in spite of symptoms and if encouragement fails ridicule and even sternness may be used. I believe all the men would recover quicker if they could be sent to the country and required to do farm work according to their ability, under supervision, for a year provided the life on the farm were hygienic. Medication is of least importance, but is necessary. The majority of the patients need iron in some form. The syrup of the iodid of iron, iron pyrophosphate, and Bland's pill, in the order named, I believe are the most useful forms. I should think that at least a year or eighteen months will be required to put the men on their feet and there will be many failures.

The problem has been well stated by Henry Kittell Webster in Mary Wollaston. Speaking of Mary's brother Rush, who has been referred to as a healthy young animal, he says "Rush, to begin with, isn't a healthy young animal. He's one of the war's sacrifices, precisely as much as if he had had his leg shot off. He needs support, will go on needing it for two or three years, financial as well as moral. He mustn't be allowed to fail. He's spent, you see, depleted. One speaks of it in figurative terms, but it's a physiological thing—if we could get at it—that's behind the lassitude of these boys. It all comes back to that. That they're restless, irresolute. That they need the stimulus of excitement and can't endure the drag of routine. They need a generous allowance, even for an occasional failure in self-command."

VARIATION OF THE PHENOLSULPHONEPHTHALEIN EXCRETION WITH THE URINE VOLUME IN CHRONIC INTERSTITIAL NEPHRITIS

R R SNOWDEN, M D,
PITTSBURGH

The phenolsulphonephthalein test is one of the best known and most reliable of the many procedures which aid in the estimation of the functional activity of the kidneys. Since its introduction by Rountree and Geraghty, it has been employed extensively and has proved to be an invaluable clinical aid. Our knowledge of the significance of the test is purely empirical, however, being based on the evidence presented by thousands of tests, the findings of which have been carefully compared with clinical, experimental and pathological examinations. Hence, any additional data which throws light on the manner of the excretion of phenolsulphonephthalein is always of interest and value.

The following studies seem to indicate that as the ability of the kidney to excrete phenolsulphonephthalein becomes impaired in many cases of chronic interstitial nephritis, the process of excretion becomes more and more one of simple filtration, and the amount of dye eliminated varies directly with the volume of urine. In their original communications, Rountree and Geraghty state that phenolsulphonephthalein is excreted independently of the amount of urine. This is true of normal kidneys as long as there is sufficient urine to wash the dye out of the urinary passages, the recovery being from 50 to 60 per cent in the first hour, an additional 10 or 20 per cent in the second hour, and only traces thereafter. It has been noticed, however, that in cases of impairment, this preponderance of the amount excreted during the first hour, over that of the second hour, diminishes, and one finds more or less equal percentages appearing. Thus, a patient may show a total two hour excretion of 60 per cent, but, of this, 30 per cent appears in the first hour and 30 per cent in the second hour, whereas in a normal case, the first hour's excretion would be 50 per cent, and that of the second hour 10 per cent. The significance of this is better appreciated if one takes into consideration not only the total amount of dye but, also, the amount of urine, or, in other words, the concentration of the dye. In the following data, the concentration of phenolsulphonephthalein is noted and certain important facts are brought out which would not be clear otherwise. For convenience of comparison, the concentration is

represented by an arbitrary figure obtained by estimation the amount of the dye in one cubic centimeter of urine. This is spoken of as the "concentration figure"¹

1 CASES SHOWING NORMAL PHENOLSULPHONEPHTHALEIN EXCRETION

Examination of the concentration figures in cases where the phenolsulphonephthalein is excreted normally, indicates that the normal percentage of the dye is excreted in the first and second hours, irrespective of the amount of urine. The concentration may be high or low, depending on the water output.

CASE 1—*Diagnosis*—Acute sinusitis

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	75	70	52.5
1-2	50	20	10
2-4	285	tr	tr

CASE 2—*Diagnosis*—Acute parenchymatous nephritis (hyperpermeability)

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	30	200	60
1-2	75	22	16
2-4	145	tr	tr

CASE 3—*Diagnosis*—Neurasthenia

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	225	23	51.7
1-2	410	21	9

CASE 4—*Diagnosis*—Pernicious anemia

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	105	50	50
1-2	330	54	18

¹ During the performance of complete renal function studies on a series of fifty hospital cases, most of which showed nephritis, the total phenolsulphonephthalein output in each specimen was calculated by determining the percentages of the dye in 1 c c urine, and multiplying this figure by the number of cubic centimeters in the specimen. This method was adopted for two reasons. First, other determinations were being made on the same specimens and only part was available for the phenolsulphonephthalein, and second, by diluting until the depth of color was such that it fell close to 25 per cent in the Helge colorimeter, it was possible to obtain more accurate and uniform readings. The procedure was carried out by diluting 10 c c of the urine specimen until the proper color was obtained, reading this in the colorimeter against the usual standard, and from this data calculating the percentage of dye in the total specimen. Thus, as an example, if 10 c c diluted to 200 c c gave a reading of 25 per cent, then 10 c c diluted to 1,000 c c would read 5 per cent, and 1 c c excreted in 1 c c. Multiplying this by the number of cubic centimeters in the specimen will give the total amount of dye excreted. It will be seen, that the percentage of phenolsulphonephthalein in 1 c c urine represents the concentration. For the sake of comparison, and to eliminate decimal points, the figures representing the concentration are multiplied by 100, and are spoken of as the "concentrated figures."

2 CASES SHOWING IMPAIRMENT OF FUNCTION AS REGARDS
PHENOLSULPHONE-PHTHALEIN

The following is a series of cases in which there is an impairment of the functional capacity of the kidney as regards phenolsulphone-phthalein. In these cases one fails to find the high concentration shown by small specimens in normal cases. It is also to be noted that instead of mere traces appearing after the second hour, measurable amounts are present, also the total output for the first hour approaches that of the second hour.

CASE 5—*Diagnosis*—Chronic nephritis, mild

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	475	10	47.5
1-2	210	7.5	15.7
2-4	250	2.7	6.7

traces during ensuing four hours

CASE 6—*Diagnosis*—Chronic interstitial nephritis, mild

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	270	12	32.4
1-2	105	14	14.7
2-4	205	3	6.1

traces during ensuing six hours

CASE 7—*Diagnosis*—Chronic nephritis, moderately severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	200	10	20
1-2	165	10	16.5
2-4	185	4.5	8.5

traces during ensuing two hours

CASE 8—*Diagnosis*—Chronic interstitial nephritis, moderately severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	240	11	26.4
1-2	100	7.5	7.5
2-4	435	2.5	10.8

traces for two hours

CASE 9—*Diagnosis*—Chronic interstitial nephritis, severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	205	6	12.3
1-2	125	12	13
2-4	100	4	4
4-6	272	2	5.4

traces during ensuing four hours

CASE 10—*Diagnosis*—Chronic interstitial nephritis, severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	30	50	15
1-2	25	50	12.5
2-4	45	35	13
4-6	35	15	5.2

traces during ensuing six hours

CASE 11—*Diagnosis*—Chronic interstitial nephritis, severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	225	10	22.5
1-2	180	10	18
2-4	210	7.4	15.5
4-6	105	5	5.25

traces during ensuing four hours

CASE 12—*Diagnosis*—Chronic and acute interstitial nephritis, severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	42	22	9.2
1-2	110	23	25
2-4	65	15	9.7
4-6	135	8	10

traces during ensuing four hours

Same case, two days later, more severe

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	42	19	7
1-2	64	20	12
2-3	15	14	2

Examination of these cases reveals the following outstanding features

First—There is universal prolongation of the time of excretion. The dye will appear in measurable quantities during a period of four or even six hours. Moreover, the recovery of the first two hours is, in the more advanced cases, materially augmented by that of the ensuing two or four hours. In the mild cases, it will be seen that the additional amounts excreted in the 2-4 hour period is about 6 per cent, in the moderately advanced cases the recovery in the ensuing four hours is from 15 to 20 per cent, sufficient to increase very materially the total phenolsulphonephthalein recovery.

Second—One fails to find the high concentration figures shown in the normal cases when the urine volume is low (Compare cases 1 and 10). Case 1 shows nearly three times the urine output in the first hour, and yet the concentration is one-third greater than in Case 10, resulting in a normal phenolsulphonephthalein excretion. During the second hour, in the normal case, the concentration drops to 20, whereas in Case 10 it remains unchanged (50). During the next two hours, in Case 1 only traces are recovered, despite the tremendous increase in urine volume, but in Case 10 the output of the dye continues steadily and is even carried over into the 4-6 hour period. Compare in the same way Case 2. The first hour urine output is of the same quantity in each case, yet the enormous concentration figure of 200 is attained in Case 2, with a resulting normal phenolsulphonephthalein total, despite the small volume of water.

Third—There are more or less parallel variations in the phenolsulphonephthalein output and urine volume. In practically all cases a

deliberate attempt was made to cause wide variations in urine output by forcing water at certain times. This was difficult to accomplish in the advanced cases because of the clinical condition. In those cases in which this attempt succeeded, it is very evident that water output does affect phenolsulphonephthalein output. This is especially true in Case 12. Compare this with normal Cases 2 and 3, in which there is the usual small phenolsulphonephthalein return during the second hour, despite the marked increase in urine volume.

3 CASES OF MARKED IMPAIRMENT

In the final stages of advanced chronic interstitial nephritis there is a total suppression of phenolsulphonephthalein excretion. The cases just short of this final stage often show traces of the dye appearing during a period as long as ten hours. The difficulties of measuring such faint traces preclude the possibility of determining the ability of such kidneys to concentrate. In Case 13, however, we find a stage in which very small amounts were being excreted, yet sufficient in quantity to be measured. Its importance is evident and it was studied carefully and is presented in detail.

CASE 13—History—Advanced chronic nephritis of two years standing, chronic edema and dyspnea for past year, severe headaches, occasional drowsiness, blood pressure, 240. Eye grounds negative. Marked arteriosclerosis. Urine from 350 to 1,500 c c daily, sp. gr. 1.010, pale, clear, acid, albumin from 0.25 per cent to 0.5 per cent with many finely granular and hyalin casts, no blood.

First test. Incoagulable blood nitrogen 98 mg per 100 c c

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	40	12.5	5
1-2	50	14	7
2-4	92	10	9.2

Second test, six days later. Patient is preuremic, being drowsy and irrational. Incoagulable blood nitrogen 160 mg per 100 c c

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	100	3.4	3.4
1-2	82	3.4	2.7
2-3	82	3.4	2.7

traces during ensuing six hours

Third test, five days later. Has been treated by bleeding, sweating, purging alkalies. Feels better, is bright and comfortable. Incoagulable blood nitrogen 98 mg per 100 c c

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	60	8.4	5
1-2	75	8.4	6.3
2-3	135	7	9.4
3-4	110	4.6	5

Fourth test, eight days later Feels fairly well Walking about
Incoagulable nitrogen 78 mg per 100 c c

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	200	25	5
1-2	155	35	54
2-4	170	36	61
4-6	145	25	36
6-8	150	2	3
8-10	175	1	17
10-12	475	tr	tr

Fifth test, one day later

Time hours	Urine c c	Concentration figure	Phenolsulphone- phthalein %
0-1	180	18	24
1-2	275	18	49
2-3	215	18	38
3-4	70	18	12

The day after this last test was made the patient left the hospital, feeling quite well, and was lost track of

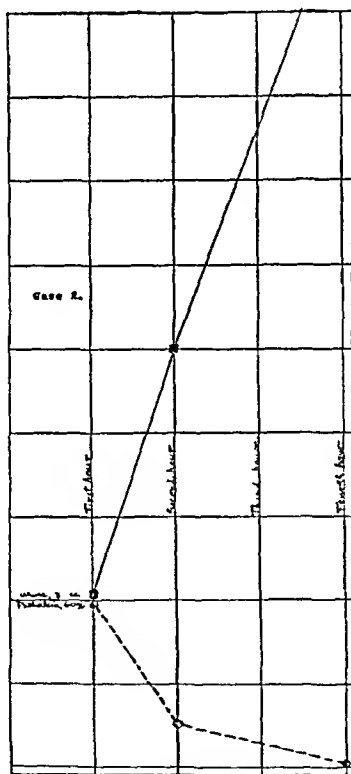


Fig 1—Case 2 Acute parenchymatous nephritis This chart and those following show relationship between the phenolsulphonephthalein output and the urine volume in various cases

In all of the tests made on this patient we find a universally low concentration of the phenolsulphonephthalein and a resulting variation in the output directly parallel with the water volume. The fact that the concentration was always low and remained practically the same throughout each test, indicates that the phenolsulphonephthalein elimination was probably dependent on the water volume, suggesting simple filtration rather than active secretion.

The charts (Figs 1 to 6) show graphically the relation between the phenolsulphonaphthalein output and the urine volume in normal cases and those with various degrees of functional impairment. In each chart the curve for the urine and the curve for the phenolsulphonaphthalein begin at the same point, and the ensuing curves are plotted so as to exhibit the elimination of each as compared with that of the first hour. Thus, for example, if the urine secretion during the first

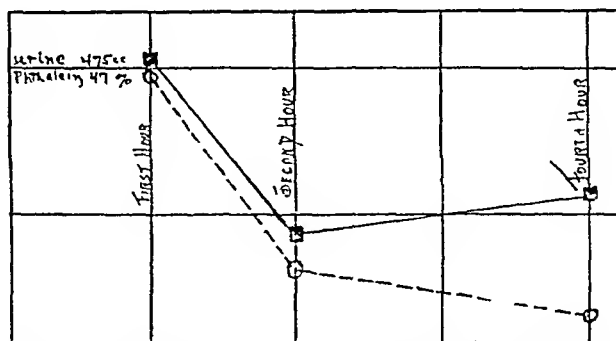


Fig 2—Case 5 Mild, chronic nephritis

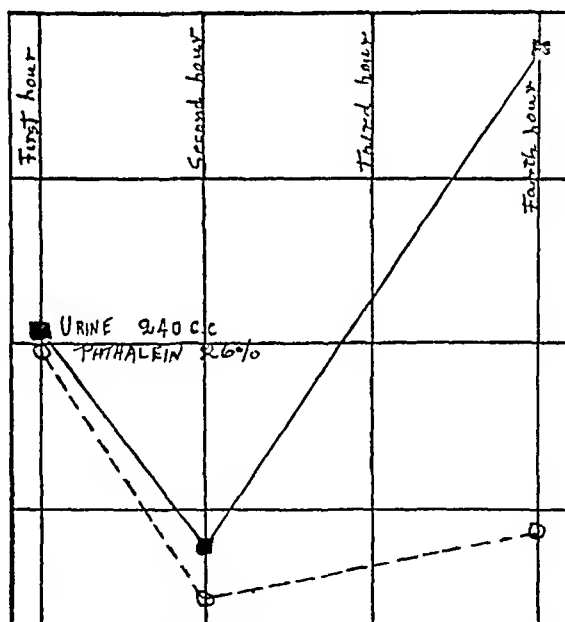


Fig 3—Case 8 Moderately severe chronic interstitial nephritis

hour is 30 c c and in the second hour is 90 c c, the elimination in the second hour is three times that of the first and the curve is so plotted, if, at the same time, the phenolsulphonaphthalein excreted in the first hour is 9 per cent and in the second hour it is 27 per cent, the excretion for the second hour is likewise three times that of the first hour and the phenolsulphonaphthalein curve, so plotted, will be parallel to that of the urine. By this plotting on the basis of the first hour's elimination, the relation between the phenolsulphonaphthalein excretion and the urine volume is most clearly depicted.

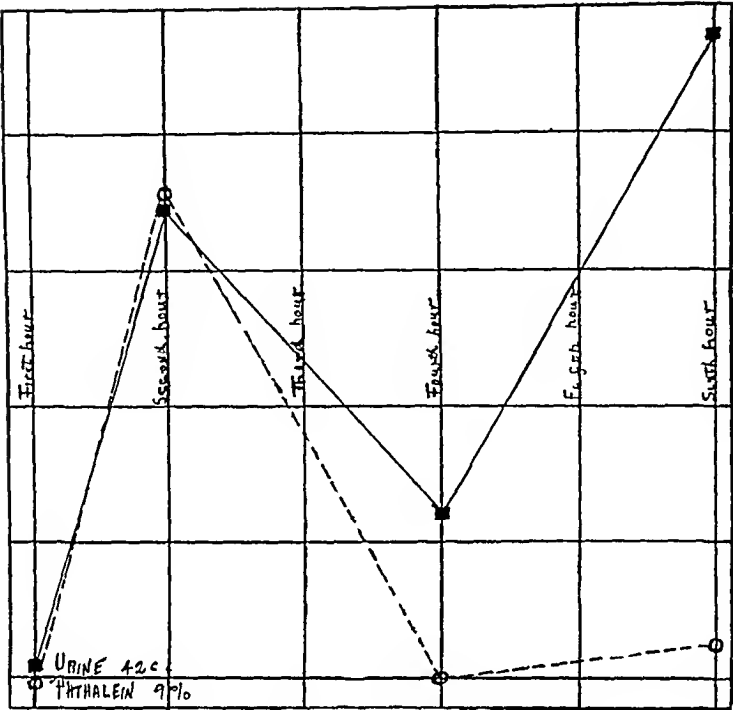


Fig 4—Case 4 Pernicious anemia

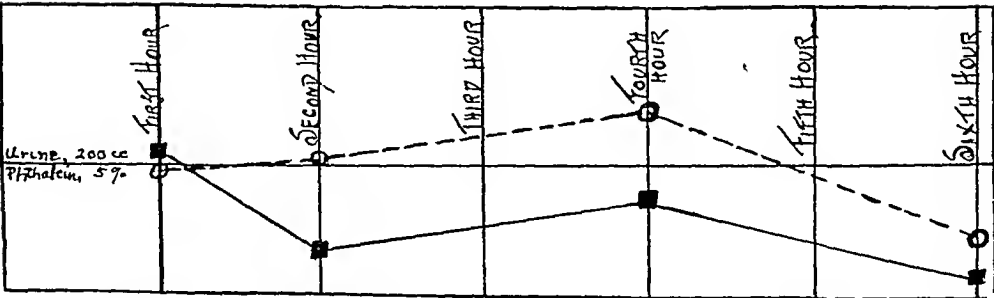


Fig 5—Case 13 Advanced chronic nephritis Fourth test

DISCUSSION

This phenomenon of excretion by simple filtration is to be observed in relatively few cases. It is not intended to give the impression that all cases of advanced nephritis show an excretion of phenolsulphonephthalein absolutely parallel to the urine volume, but it is plain that most cases of chronic interstitial nephritis do exhibit that tendency, as evidenced by prolongation of excretion and the recovery of greater amounts of the dye when the urinary output is stimulated by forcing water. Consequently, one is justified in asserting that in cases of chronic interstitial nephritis, with impairment of function, the elimination of phenolsulphonephthalein is materially increased when the urinary flow is stimulated by forcing water.

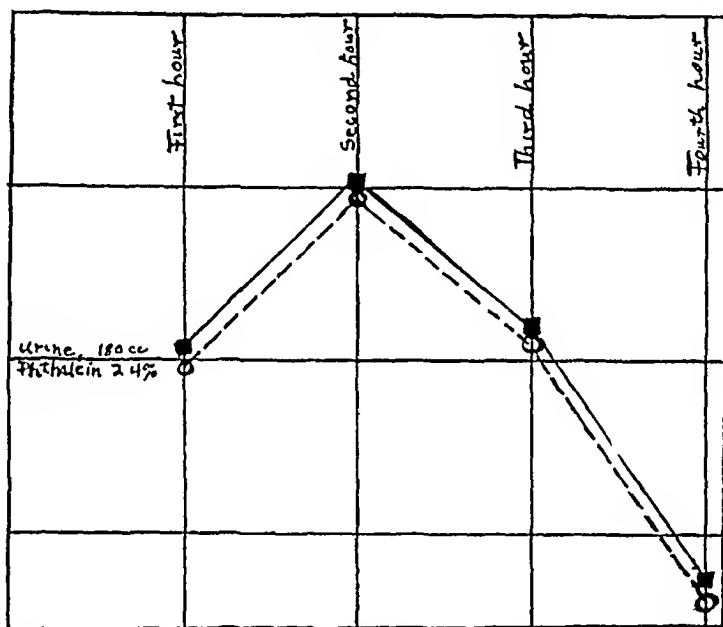


Fig 6—Case 13 Advanced chronic nephritis Fifth test

The physiologic explanation of this phenomenon remains to be determined. It is likely that in all cases the phenolsulphonephthalein is excreted by both active secretion and passive filtration. In the normal cases the latter is completely obscured by the former, but as damage of renal cells eliminates the active secretion, the filtration part becomes more and more apparent.

Whatever the explanation may be, the actual observation remains that the forcing of water will materially increase the elimination of the dye in cases of marked functional impairment. The knowledge of this fact is important for two reasons. In the first place, when drawing deductions from a phenolsulphonephthalein test in which the amounts of the dye eliminated are much reduced, the volume of urine should always be considered. In the second place, since the phenolsulphonephthalein is eliminated by the same mechanism as nitrogenous

waste products and is a measure of the ability of the kidney to excrete nitrogen, the data here presented indicates that it is of clinical value to stimulate urinary flow as an aid to elimination in cases of nephritis showing a retention of nitrogenous waste products ²

CONCLUSIONS

1 In cases of chronic interstitial nephritis, showing functional impairment as regards phenolsulphonephthalein, there is a retardation and prolongation of the excretion of the dye

2 This retardation is the earliest indication of functional disability

3 As impairment becomes more marked the retardation becomes more pronounced

4 In most cases of marked functional impairment, the excretion of the phenolsulphonephthalein varies more or less directly with the volume of urine, and in these cases the phenolsulphonephthalein output is materially increased when the flow of urine is stimulated by giving water

2 Many cases of chronic interstitial nephritis exhibit concomittant conditions which, of course, contraindicate the forcing of water. Cardiac insufficiency, chlorid retention with resulting anasarca, etc, may one or all so complicate the condition that the ingestion of large amounts of water would do more harm than good

RELATION OF THE GASTRIC CONTENT TO THE SECRETORY AND MOTOR FUNCTIONS OF THE STOMACH *

HOMER WHEELON, M D
ST LOUIS

From the time of Spallanzani, who was among the first to demonstrate the digestive action of gastric juice, to the present day gastrologist, wide variations of opinion have been held relative to the secretory and motor powers of the stomach. The lack of agreement among students of gastric functions recalls the statement of Hunter "Some Physiologists will have it that the stomach is a Mill,—others, that it is a fermenting Vat,—others, again, that it is a Stew-pan—but in my view of the matter, it is neither a Mill, a fermenting Vat, nor a Stew-pan—but a *Stomach*, Gentlemen, a *Stomach*."

The "fractional method" of gastric analysis, now so popular among certain clinicians, is based on the assumption that the gastric content is of a homogenous nature. The method also infers that the determination of the acidity of gastric samples removed at stated intervals, more or less, directly show the degree of acid formation by the stomach and that any such sample is indicative of the acid concentration of the entire stomach contents at the time of withdrawal. As stated by Gorham,¹ "If the above hypothesis be a correct one, and based upon a true physiological principle, then we should expect the acid concentration of the different portions of the remaining gastric chyme (*content*) (Italics are mine) to be similar at these different intervals after a test meal." That such is not the case has been well shown by this observer. To quote "This hypothesis is not based upon true physiology. The acidity of one portion, as obtained by the fractional method, may differ widely from the acidity of different portions of the remaining contents. In the so-called "fractional," or other methods of gastric analyses when only a small sample is withdrawn, the small portion removed may or may not be representative of the gastric contents remaining in the stomach."

It is my purpose to call attention to certain observations concerning the relation of gastric juice to the ingested food and to present experimental data which corroborates the views of certain of the older

* From the Department of Physiology of the St. Louis University School of Medicine

1 Gorham, F. D. Variations of Acid Concentration in Different Portions of the Gastric Chyme, and Its Relation to Clinical Methods of Gastric Analysis, Arch Int Med **27** 434 (April) 1921

observers and especially of the recent work of Gorham, namely, that the stomach content is not a homogenous or uniform mixture during the greater portion of the period of gastric digestion

METHODS

The results here reported are based on 296 gastric sample titrations from sixty-four normal medical students. In addition, gastric analyses were made on three men who did not show the presence of free hydrochloric acid. After a thorough examination of the sixty-four men, it was felt that the results obtained could be taken as representative of the average normal for the young male.

In the present work three methods of procedure were employed in determining the acid secretion of the stomach. These methods were (1) The usual "one hour test" following complete removal of the stomach contents at one time. (2) The "fractional method" or "acid functional test" in which withdrawals of gastric contents were made every fifteen minutes following the ingestion of a test meal. (3) A method recently described by Gorham which consists, in principle, of rapidly removing the entire contents of the stomach in 10 c c amounts at a stated time following the taking of a test meal. The third method is here referred to as the "rapid withdrawal method, or test."

In Method 1, the gastric contents was obtained by the use of the large stomach tube at the end of one hour from thirty-one men. In the other two methods a small, soft, stomach tube of the Rehfuß type was passed, the fasting contents removed, and then, with the tube remaining in the stomach, the subjects were given a test meal. In Method 2, "fractional," 10 c c quantities of gastric content were withdrawn every fifteen minutes following completion of the meal until the stomach was emptied. This method was used on twenty men, 148 samples were obtained and titrated. In the third method, "rapid withdrawal method," nineteen men were given the tube, and one hour after the test meal the stomach was emptied as rapidly as possible by repeated withdrawals of 10 c c amounts. The samples were titrated separately. The nineteen men on whom this method was used returned a total of 117 samples.

Inasmuch as the passage of a stomach tube for the first time is usually associated with more or less retching, salivation, elevation of the blood pressure and nausea, it was deemed advisable to introduce the tube in the second and third methods prior to the ingestion of the meal. Further, it was hoped that such a procedure would permit the tip of the tube to descend into the pyloric portion in which region the concentration of acid is conceded to be highest and the gastric content of a more uniform consistency. That is, an attempt was made to

obtain results as uniform as possible and to reduce to a minimum the variations in the acid concentration due to withdrawals from various portions of the stomach

The test meal used throughout this series of experiments consisted of two slices of white bread and 500 c c tap water. The meals were given at 8 a m or following a fast of from ten to twelve hours. The

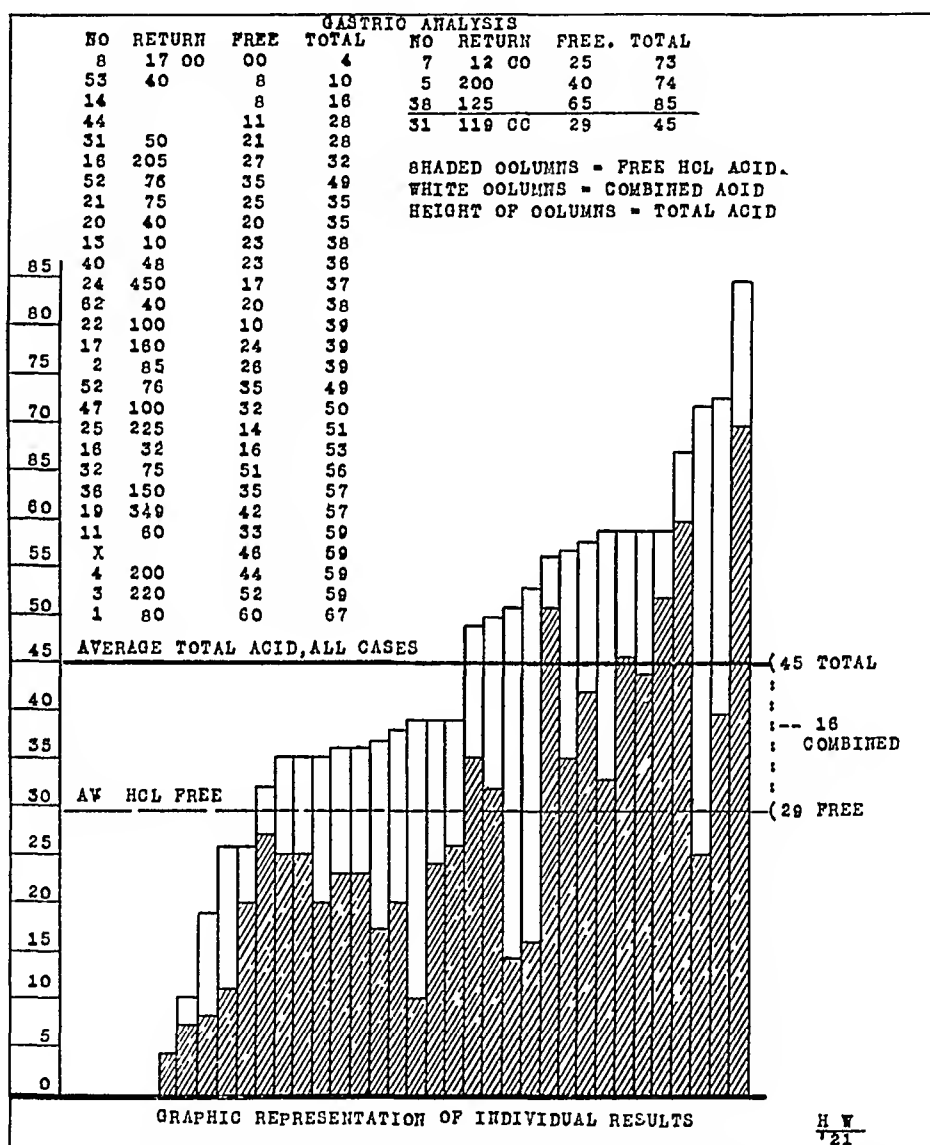


Fig 1—Chart showing the individual amounts of gastric return and free and total acids at the end of one hour following the ingestion of a standard test meal. Observations made on 31 young men.

contents of the fasting stomach averaged less than 30 c c. The acidity of the gastric contents was determined by the Toepfer method of gastric analysis, titrating with tenth-normal sodium hydroxid solution. Dimethylamidoazobenzol and phenolphthalein were used as indicators. Titrations were made on 10 c c samples of gastric content.

RESULTS

Results of Experiments by the "One Hour Method"—In these experiments the entire gastric content was received one hour after ingestion of the test meal into a single container. This material was measured then thoroughly mixed and a 10 cc sample titrated for free and total acids. The results obtained by such a procedure represent the degree of acidity of the mass as a whole, also the degree of gastric evacuation that had occurred during the hour.

The results of thirty-one experiments of this nature are shown numerically and diagrammatically in Figure 1, in which the data are arranged according to the amounts of total acid found. The average total acids for the thirty-one titrations was 45, free acid, 29, combined acid, 16. The range of total acid variation among the thirty-one determinations was from 4 to 85, free hydrochloric acid varied from 0 to 65. The average figures for the entire number of tests lay within the range of "the normal" as generally accepted. It is possible that the first three men of the series show a reduced secretory power of the stomach, however, none of the three had experienced gastric disturbances. Student 8 failed to show the presence of free acid by any one of the three methods employed, also his total acid was continually low.

The average gastric return for twenty-eight men of this group was 119 cc. The range of variation in returns was great, from 10 to 450 cc. This point will be considered further in relation to gastric motility.

Results of Experiments by the "Fractional Method"—This method of gastric analysis was studied on twenty-seven men who gave a total return of 183 samples. Of this number of men, twenty were given a test meal and withdrawals were made every fifteen minutes until the stomach was emptied. In seven cases, the men took the tube following a regular breakfast of fruit, cakes, water and coffee. The results on these seven men, while differing but little from those obtained on the group of twenty men, are incomplete for the reason that withdrawals were begun at various time-intervals following the taking of the meal (Table 1).

The numerical results of the observations by the "fractional method" are shown in Table 1, and graphically in Figures 2 and 3. In Table 1 the results are arranged according to the number of samples returned at the various fifteen minute intervals. Of this group of twenty men, one returned fourteen samples—210 minutes, one returned eleven samples, two returned ten samples, two returned nine samples, one returned eight samples, four returned seven samples, four returned six samples, and five returned five samples. The average time of gastric evacuation

for the group was 111 minutes, or an average return of 7.4 samples. The results of titrations on any one man are shown in columns, Table 1, under the heading Case No. The comparative results for any given time interval are shown by the figures to the right of the time intervals, from 15 to 210 minutes. Averages are shown both for the individual

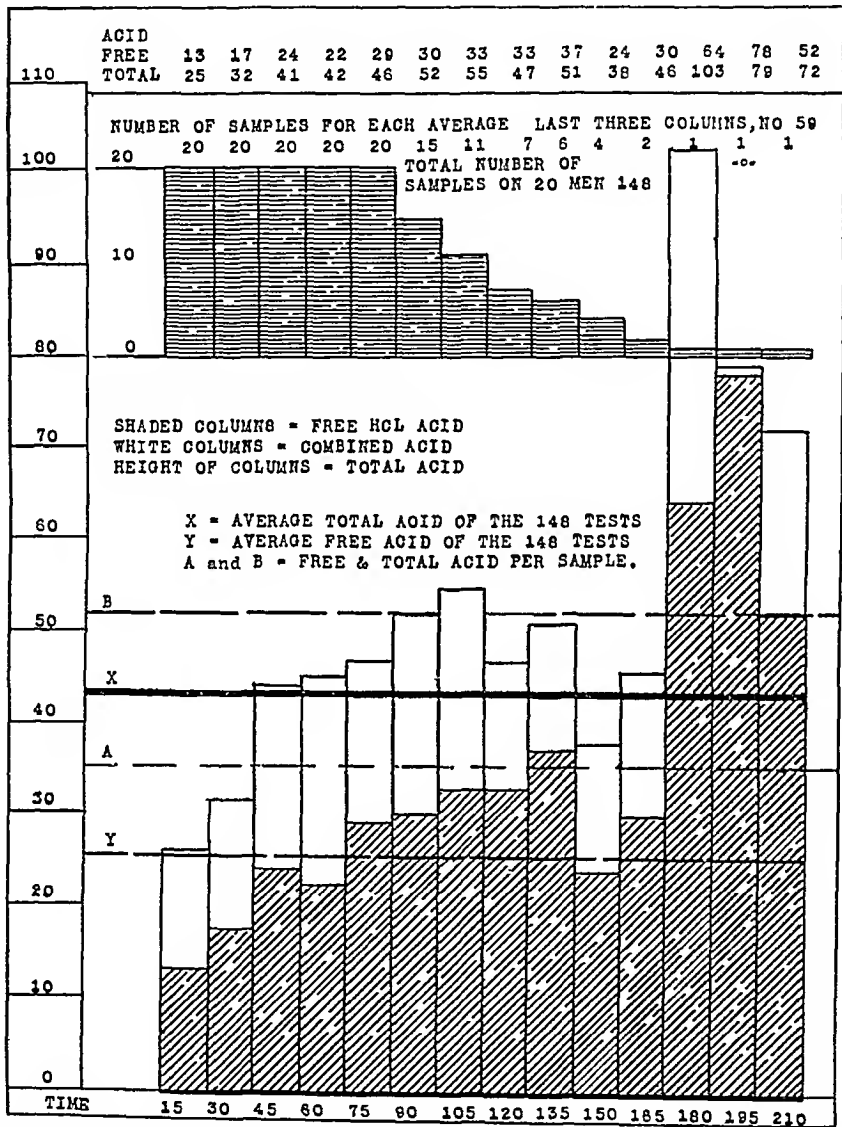


Fig 2—Chart showing the collective average of free and total acid for the entire number of 10 c.c. samples delivered at 15-minute intervals. "Fractional test meal method."

case and for the collective readings at the various time intervals. The range of acid variation is also shown in Table 1.

The relation of the average of all readings for the group of twenty men for any given time period to the number of samples examined is shown in Figure 2. Here, it will be seen that the average free acid for the number of men delivering samples shows a gradual increase up

to the 135 minute period following the test meal, also that the total acids increase only up to the 105 minute period. The last three columns are the results obtained on one man (No 59), and, therefore, cannot be taken to indicate a terminal high acid concentration for the group, as a whole. The average free and total acid concentrations for the fourteen time intervals was 35 and 52, respectively, the average for the entire number of 148 titrations was free acid 25, total 45. The average variation for acid determinations made on any one individual was free

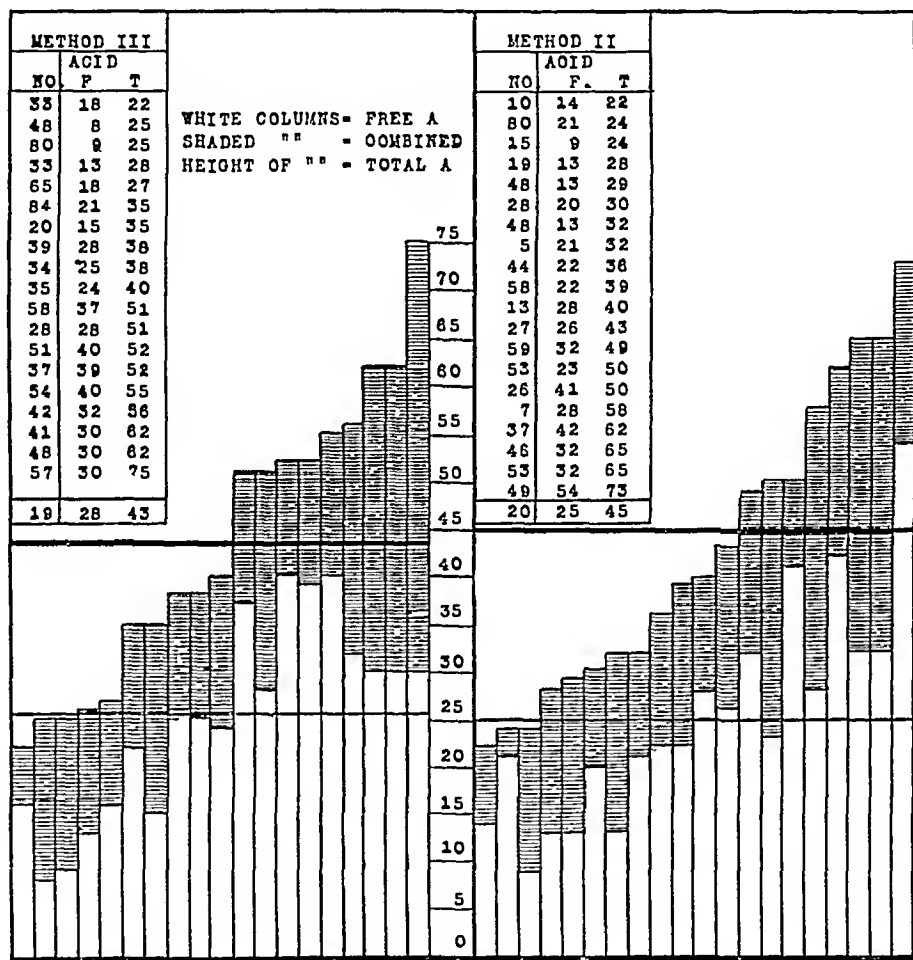


Fig 3—Chart showing the individual average free and total acid of the entire number of samples of gastric contents removed in 10 c.c. quantities at the end of one hour "rapid withdrawal method" and at intervals of 15 minutes following a test meal "fractional method"

acid 34, total 41 points. The average variation among titrations for any given time interval was free acid 53, total 67 points.

In Figure 3 are shown the average free and total acid concentrations for the entire number of samples obtained from any one man. The figures are arranged according to the average total acid concentrations. The difference in the height of the twenty columns indicates the range of variation in the average acid concentrations among the various

members of the group It is of interest to note that the average figures for the entire number of titrations for this group of twenty men made over a period of 210 minutes correspond closely with the averages obtained for the first method—total acids 45 against 45, and free acid 25 against 29 This appears to be of interest since the height of free acid concentration, as determined by the second method, occurs 135 minutes following the meal, whereas the acid concentration in the first method was determined at the close of one hour Such results seem to indicate that as much information relative to the secretory power of the stomach is to be gained by the "one hour method" as from the more laborious procedure of the "fractional method"

Results of Experiments by the "Rapid Withdrawal Method"—The primary purpose of this method, as evolved by Gorham,¹ is the determination of the presence or absence of free acid in any portion of the gastric content at the end of a given period of time Such a method often reveals the presence of free acid in some one or more of the samples when other methods have failed to show its presence However, three of the sixty-four students studied, Nos 63, 29 and 8, did not show the presence of free acid by the first two methods, also failed to show free acid by the rapid withdrawal method It, therefore, appears that these men had a condition of true achlorhydria

The reason for the use of this method, as modified for the purpose of this study, was, not to search for free acid in patients, but, simply to determine whether or not the degree of acidity in various portions of the gastric content in normal individuals was uniform, and to compare such results with those obtained by the other two methods The results of such observations on nineteen normal young men—117 titrations—are shown numerically in Table 2 and graphically in Figure 3 The data in Table 2 is arranged as in Table 1 In this series of observations two men delivered but two samples (20 c c) at the end of one hour, three delivered five, nine delivered six, two delivered seven and three men delivered, 9, 10 and 11 samples, respectively The average free and total acids for the series of eleven samples, as shown in Table 2, is fairly constant save for the last two columns—samples 10 and 11 This discrepancy is explainable on the basis of the number of men delivering ten and eleven samples The average figures for the series of the eleven collective titrations was free acid 26, total acids 41 The average figures for the entire number of titrations (117) was free acid 26, total 43 The average range of variation among the eleven sets of samples was free acid 34, total acid 53 points The average range of variation for the total number of samples delivered by the individual case was free acid 16 points, total 25 The maximal and minimal variations in the acid concentrations are shown in Table 2

TABLE 2—SHOWING THE RESULTS OF RAPID EMPTYING OF THE STOMACH AT THE END OF ONE HOUR BY SUCCESSIVE WITHDRAWALS OF 10 cc AMOUNTS

Sample	Free Hydrochloric Acid Present																										Absent										
	Case Number																										Case Number										
	Acid	33	54	46	37	26	34	35	39	42	51	57	60	56	20	33	41	64	48	65	No	Av	Max	Min	Dif	63	29	8	Av								
1	F	14	52	22	30	18	25	23	25	39	40	29	11	37	11	26	42	17	6	16	19	25	52	6	46	00	00	00	00								
2	T	44	65	48	40	37	43	45	43	53	52	68	30	52	20	39	54	25	18	28	19	42	63	18	40	15	8	8	10								
	F	20	40	28	40	22	19	20	31	42	38	27	14	35	4	20	53	17	10	17	19	27	53	4	49	00	00	00	00								
3	T	27	67	44	55	45	40	51	41	55	53	66	36	51	13	33	61	30	32	26	19	43	67	13	44	8	14	10	11								
	F	14	42	19	50	41	26	27	24	45	40	26	3	38	15	12	51	25			17	20	51	3	48	00	00	00	00								
4	T	24	80	56	63	70	48	49	46	56	51	65	24	49	42	28	61	43			17	50	80	24	56	3	12	20	12								
	F	12	50	23	45	30	16	19	14	41	27	10	39	14	3	45	22			17	25	50	3	47	00	00	00	00	00								
5	T	12	57	46	75	66	19	23	23	57	53	64	20	52	42	21	58	42			17	44	75	12	63	3	12		8								
	F	17	30	25	35	21	30	30	31	45	37	34	10	37	19	2	47	26			17	98	43	2	43	00	00	00	00								
6	T	18	32	50	45	38	39	37	53	53	34	82	22	50	45	8	78	36			17	12	83	8	74	7	13		10								
	F	19	35	40	33	34	32	33	45	41	35	2	38	28						14	31	45	3	42	00	00	00	00	00								
7	T	22	51	77	46	45	39	34	40	56	51	104	16	50	47					14	40	104	16	88	5	12		8	8								
	F	21	45	48	30	32														5	35	48	21	27	00	00	00	00	00								
8	T	24	64	90	40	55														5	35	48	21	27	66	3			9								
	F	18	53	20																3	32	58	24	60	40												
9	T	24	74	42																3	47	74	18	50	40												
	F	15	23	42																3	27	42	24	27	60	7	13		10								
10	T	20	32	107																3	27	42	24	27	60	7	13		10								
	F	14	26																	2	53	107	20	87	00	00	00	00	00								
11	T	18	30																	2	20	26	14	12	00	00	00	00	00								
	F	7	8																	1	7	7	18	12	12	12	12		10								
Av No	11	10	9	7	7	6	6	6	6	6	6	6	6	6	6	5	5	5	2	2	117	11	11	10	11	7	6	3	16								
Av F per sample	11	10	9	7	7	6	6	6	6	6	6	6	6	6	6	5	5	5	2	2	11	26	43	9	34				00								
Av T per sample	11	10	9	7	7	6	6	6	6	6	6	6	6	6	6	5	5	5	2	2	11	41	71	18	53				00								
Per F	16	40	30	39	28	25	24	26	33	40	30	9	37	15	13	48	21	8	16		26	26	43						7								
Man T	22	55	62	52	51	38	40	38	56	52	75	25	51	35	26	58	35	25	27		43																
Variations																																					
Free Max	21	58	48	50	41	34	30	33	45	41	35	14	39	28	26	53	26	10	17		34								00								
Acid min	7	23	19	30	18	16	19	14	14	37	26	3	35	4	2	42	17	6	16		18								00								
Dif	14	35	29	20	23	18	21	19	31	4	9	11	4	24	24	11	9	4	1		16								00								
Total max	44	80	107	75	70	48	51	46	58	53	104	36	52	47	39	61	43	32	28		57								16								
Acid min	8	30	42	40	37	19	23	23	53	51	64	16	49	13	8	54	25	18	16		32								6								
Dif	36	50	65	35	33	29	28	23	5	2	40	20	3	34	31	7	18	14	2		25								10								
Greatest individual variation																										F 35 No 54 T 65 No 46				Minimal individual variation				F 1 No 65 T 2 No 65 and 71			
Greatest variation in any set of samples																										F 49 Sam 1 T 88 Sam 6				Minimal variation in any set of samples				F 12 Sam 10 T 12 Sam 10			

F 1 No 65
T 2 No 65 and 71
F 12 Sam 10
T 12 Sam 10

Minimal individual variation
Minimal variation in any set of samples

F 35 No 54
T 65 No 46
F 49 Sam 1
T 88 Sam 6

In Figure 3 is shown the average free and total acids for the entire number of samples obtained from any one individual. The average free and total acids for the 117 titrations made on nineteen men, as stated above, was 26 and 43, respectively. As shown in this figure, the individual averages for the entire gastric return show a greater range of variation than those representing the averages for any one particular set of samples. That is, the range of variation among the total number of titrations for the individual is greater than the variation for the collective average of the eleven sets of samples. The results of gastric analysis by this method is strikingly similar to those obtained by the "one hour method." Probably, as great variations in the acid concentration of the gastric contents would have been shown for each individual by the first method as by the third had the returned mass been divided into portions, titrated and the average ascertained. In a way this third method is simply a modification of the "one hour method," hence, it is not surprising to find that the average acid values as determined by the two methods are so closely similar. However, the point of interest for this method lies, not in the obtaining of average figures, but in the fact that by its use the acid concentration in the various portions of the stomach content can be ascertained. The degree of variation in the acid concentration of the samples returned by an individual at the end of one hour is shown in Table 2. The average variation among all samples was free acid 16, total 25 points. The maximal variation for any one individual was free acid 35, total 65, the minimal variation was free acid 1, total acid 2 points. The range of variation among individuals, therefore, was free acid 1 to 35 and for total acid 2 to 65 points. In other words, different individuals show marked variations in their power of reducing a test meal to a uniform degree of acidity in the course of one hour.

The amount of material recoverable from the stomach at the end of an hour seems to bear no constant relation to the degree of acidity of the various samples. The above figures do not indicate that the last samples withdrawn are of necessity more charged with acid than the first withdrawals, although a number of cases studied did show such a terminal increase of acid, Nos. 57 and 46. On the whole, the results of the figures as obtained by the third method show that the gastric contents at any one time may not be a homogenous mass, the acid concentration varies in different portions of the mass as a whole.

The present findings on normal men are in accord with those of Gorham who used this method of study on sixty-five patients with varied clinical diagnoses. This observer found that 73 per cent of his cases showed a considerable variation between the first and subsequent portions withdrawn from the stomach forty-five minutes

following a test meal In Table 2 are shown numerically the variation in the acid values for the various portions of the returned gastric contents as determined in my series of normal young men

Motility of the Stomach as Judged by the Amount of Gastric Return—Roentgenographic procedures, to a large extent, have eliminated the necessity of indirect observations relative to the emptying of the stomach However, a careful gastric analysis, to a considerable degree, is indicative of the power of the stomach to rid itself of contents

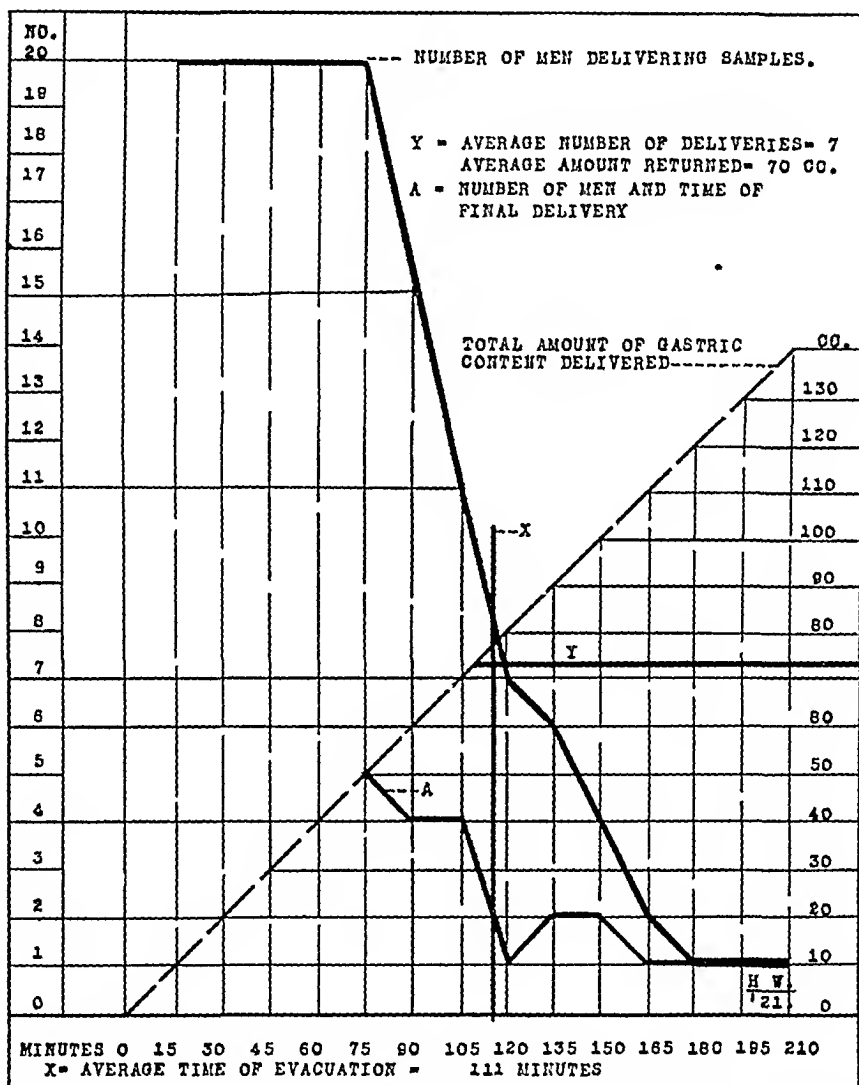


Fig 4—Chart showing the rate of gastric evacuation as determined by fractional withdrawals (10 cc) at 15 minute intervals Withdrawals made every 15 minutes following ingestion of standard test meal Observations on 20 men, 148 deliveries over a total period of 210 minutes

The results of the present observations on this point are shown in Tables 1 and 2, and in the charts In Group 1, twenty-eight men returned an average of 119 c c of gastric contents, or 23.8 per cent of the amount ingested (Fig 1) In other words, at the end of one hour, the twenty-eight men has passed into the duodenum 10,668 c c, or an

average clearance of 381 c c, or 76.2 per cent. In Group 3, nineteen men passed during the period of one hour, 8,330 c c of a total of 9,500 c c into the duodenum, average clearance of 438 c c per man, or 87.6 per cent. That is, each man delivered on the average 62 c c, i.e., 12.4 per cent, at the end of one hour. These figures show a wide variation in the amount of material that may be recovered at the end of an hour by the two methods. It is possible that in Group 1 certain men did not refrain from taking fluids prior to the test meal, also in this group the fasting content was not obtained prior to the meal. In Group 3 the fast-contents were removed prior to the meal. It is of interest to note, however, that the average free and total acids for the two groups are practically identical.

The twenty men of the second group were given a total of 10,000 c c of a fluid mixture and all of this, save the amount returned by aspiration, was permitted to enter the duodenum. Of this amount of fluid given 1,480 c c were withdrawn as samples, that is, each man averaged a return of 74 c c, or 14.8 per cent of the amount taken. This means that 85.2 per cent, or 426 c c, of the material passed into the duodenum in an average period of 111 minutes. These figures when compared with those from Group 3 seem to indicate that successive withdrawals over a period of 111 minutes, 74 c c, is the equivalent of the amount of material in the stomach at the end of one hour. In other words, successive withdrawals appear to lengthen the time for a given amount of material to pass the pylorus. This point is further emphasized by figures of Group 2, calculated for a sixty minute period. The twenty men of this group returned 800 c c in samples, that is, each man averaged 40 c c in returns, or 8 per cent. This means that 9,200 c c, or 92 per cent, of the original 10,000 c c given was either in the stomach or passed into the duodenum at the end of one hour. If these figures can be compared with those in Groups 1 and 3, then it appears that successive withdrawals during the hour material is within the stomach, in some way, reduces the power of the stomach to rid itself of contents. That as much material does not leave the stomach when the "fractional method" is employed is further indicated by the fact that only in an average of 111 minutes, practically two hours, is the stomach entirely empty and at which time 14.8 per cent of the material given has been withdrawn as samples. This percentage of withdrawals for 111 minutes is practically the same as the amount of material withdrawn at the end of sixty minutes by the third method. On the other hand, the average returns for both Groups 2 and 3 are less than for Group 1. It is possible that the anticipation of the tube by the first group in some way interfered with gastric evacuation. In Groups 2 and 3 this possible psychic factor was removed in that the tube was taken before the meal.

The data presented above, relative to gastric clearance offers no direct evidence as to the mixing power of the stomach. However, the results of the "rapid withdrawal method" do, in a rough way, indicate the degree of acid concentration in various portions of the stomach contents. It further appears from the present work, and that of Gorham¹ that gastric peristalsis does not act in such a manner as to cause any extensive mixing of the gastric contents as a whole. On this point the physiologist appears to hold a firmer view than the clinician who, by performing a "fractional" gastric analysis, presumes that the stomach contents is of a uniform acidity for the various phases at which samples are withdrawn for titration. Such gastric tests, though highly instructive if correctly interpreted, must be interpreted in the light of what really occurs when a sample of material is withdrawn from the stomach.

REVIEW OF LITERATURE

The modern views relative to the major activities of the stomach are well set forth by Rehfuess². To quote, page 28

During the digestive phase, the two parts, the fundus and the pyloric antrum, can be readily delineated. The fundus serves as a reservoir, receiving the food and pouring out the active gastric secretion but playing little part in active motor function. This accounts for the continuance of salivary digestion in the interior of the food mass for some time after food has left the mouth, and it explains the difference in the character of the material found in the fundus and duodenum on double gastro-duodenal intubation.

This author, after briefly reviewing the theory of "the acid control of the pylorus" and in the main, accepting it, states that material is regurgitated into the stomach from the duodenum whenever the acidity of the chyme in the former reaches a high degree of concentration. To quote him on this point, pages 32-33

(1) Trypsin is found almost constantly in both the fasting and the digestive phases of digestion, the interdigestive and digestive phases of the stomach. (2) Trypsin is highly resistant to the action of acid and pepsin and forms the index for pancreatic regurgitation. (3) The tryptic value was found to be high in the presence of low acidities and low when the contents were of high concentration. When normally the acidity reaches excessive limits, its fall is accomplished by a rise in the trypsin values. (4) We were able to demonstrate for instance, that 0.5 per cent HCl is followed by a fall in acidity to about 0.2 per cent due to the regurgitation of alkaline intestinal contents as indicated by a fall in acidity coincident with a rise in trypsin values.

For the purpose of this paper, the quotations stated above indicate that the acidity of the stomach content varies in different portions, being

1 Gorham F. D. Variations of Acid Concentration in Different Portions of the Gastric Chyme, and Its Relation to Clinical Methods of Gastric Analysis, *Arch. Int. Med.* **27** 434 (April) 1921.

2 Rehfuess, M. E. Diseases of the Stomach. Oxford Medicine, Oxford Univ. Press. Chapt. 2, pp. 19-121.

alkaline in the fundus and inner mass of the food, and that an accumulation of acid in the pyloric portion is neutralized more or less completely at various times by the regurgitation of duodenal contents. Just what influence these factors have on the interpretation of functional secretory curves, "fractional method," is not stated by Rehfuß although considerable attention is given to a consideration of the secretory powers of the stomach in relation to diseased conditions.

In 1814, Home³ described the dog's stomach as consisting of two portions—the first, or fundic portion, containing fluid and solid material, the second, or pyloric portion, containing half digested food of uniform consistence. Eberle, in 1834,⁴ made similar observations and further demonstrated that, when the stomach is carefully opened during digestion, the surface of the mass in the cardiac end shows signs of digestion, whereas the interior of the mass remains unchanged.

Ellenberger and Hofmeister,⁵ Ellenberger and Goldschmidt⁶ and later Scheunert⁷ showed that the digestive processes in the two ends of the stomach of the horse and pig are different for several hours after eating, also that different foods fed successively are not found uniformly mixed but in strata. Cannon,⁸ 1898, by mixing bismuth subnitrate with different portions of a meal found that the various portions of the meal when eaten were arranged in layers in the stomach. The first portion of the meal arranged itself along the greater curvature, the third portion along the lesser curvature and the second portion between the other two. Ten minutes after peristalsis began, the stratification had entirely disappeared toward the pyloric end while, after a period of eighty minutes, the layers were still visible in the fundic portion.

Grutzner,⁹ 1902, after feeding a divided meal of various foodstuffs, killed the animals (frogs, cats, dogs) at various periods of time following the meal, froze the stomachs and found that only the outer layers of food were acidified and digested. The central layers in the cardiac end retained for hours a neutral or weakly alkaline reaction. Results of a similar value were obtained by Cannon¹⁰ after feeding alkalinized meals to cats and dogs.

3 Home Lectures on Comparative Anatomy, London, 1814, **1**, p 140

4 Eberle Physiologie der Verdauung, Wurzburg, 1834, pp 81, 91, 100, 154

5 Ellenberger and Hofmeister Arch f wissensch u prakt Thier **8** 1882, **9** 1883, **10** 1884, **12** 1886

6 Ellenberger and Goldschmidt Ztschr f physiol Chem **10** 384, 1886

7 Scheunert Arch f d ges Physiol **114** 64, 1906

8 Cannon, W F The Mechanical Factors of Digestion, New York, 1911, Longmans, Green & Co, p 227, Am J Physiol **1** 378, 1898

9 Grutzner Arch f d ges Physiol **106** 463, 1905, Deutsch med-Ztg No 28, 1902

10 Cannon, W B Am J Physiol **1** 379, 1898

Knox,¹¹ in discussing the processes of gastric filling for man as observed radiographically, refers to the work of Dr Jefferson as follows

When a small quantity of opaque food in the form of an emulsion is swallowed, it may be seen at the lower end of the esophagus, where it is momentarily held up. It then flows obliquely towards the left, where it accumulates to form a triangular-shaped shadow, with the apex pointing downward. This triangular mass continues to increase as the patient swallows more food, the time elapsing before the food passes lower down seeming to depend upon the tonicity of the stomach walls. In the next stage a narrow streak of opaque food passes down into the sinus, forming a longitudinal band filling the *canalis gastricus*, which lies along the lesser curvature. This stage may last only a few seconds. The sinus is gradually distended widening out from below upwards, until the whole organ is filled, except the air space at the fornix.

Modern physiology, as exemplified by textbooks, teaches that the fundic portion of the stomach exercises a steady pressure on its contents in consequence of which successive portions of the undigested food mass are presented to the walls of the stomach to replace the layers which loosened by the digestive action of the gastric juice are swept on to the pylorus by the peristaltic waves.⁸ An older view of the movements of the gastric contents was that described by Beaumont¹² who considered that the contents of the stomach were kept in a rotary movement so as to become thoroughly mixed or churned. Possibly, the erroneous phrase "churning action of the stomach" is to be ascribed to the description by Beaumont.

Sick,¹³ found that materials withdrawn from the two portions of the stomach possessed different chemical and physical properties. He further found that coloring matter, charcoal or carmine, when taken after a semifluid meal did not appear in the pyloric region until from twenty-five to forty minutes later.

The above citations are sufficient to establish the fact that the gastric contents are not, as a rule, uniform mixtures throughout the various stages of digestion. However, I wish to call attention to several early observations which, though a century old, are in the main identical with our present theory of gastric activity.

A. P. W. Philip,¹⁴ refers to his observations on the activity of the stomach as observed in about 130 rabbits immediately after they had been killed.

11 Knox, R. *Radiography and Radiotherapeutics*. New York, 1919. The Macmillan Co., pp. 328-329.

12 Beaumont, W. *Physiology of Digestion*, Plattsburgh, 1833, p. 110.

13 Sick. *Deutsch Arch f klin Med* **98** 199, 1906.

14 Philip, A. P. W. *A Treatise on Indigestion and Its Consequences*, Etc. Philadelphia, 1822, Benjamin & Thomas Kite, p. 205.

The first thing that strikes the eye on examining the stomach of rabbits which have lately eaten is, that the new is never mixed with the old food. The former is always found in the center, surrounded on all sides by the old food, except that on the upper part between the new food and the smaller curvature of the stomach there is sometimes little or no old food. All around, the nearer the food lies to the surface of the stomach the more it is digested. This is true even with regard to the food in the small curvature, compared with that nearer the center, and the food which touches the surface of the stomach is more digested than any other found in the same part of the stomach, but, unless the animal has not eaten for a great length of time, the food in contact with the surface of the stomach is in very different stages of digestion in different parts of the organ. It is least digested in the small curvature, more in the large end, and still more in the middle of the great curvature. The foregoing observations apply to the cardiac portion of the stomach, the food in the pyloric portion is always found in a state very different from that just described. It is more equally digested, the central parts differing less from those which lie near the surface of the stomach. It is evident however, that all the change effected in the stomach is not complete when the food enters this portion of it, because we find it the more digested the nearer it approaches to the pylorus, where, being ready to pass into the intestine, it has undergone all that part of digestion which is performed in the stomach. It appears, that in proportion as the food is digested it is moved along the great curvature, where the change in it is rendered more perfect to the pyloric portion. Thus the layer of food lying next the surface of the stomach is first digested, and in proportion as this undergoes the proper change, and is moved on by the muscular action of the stomach, that next in turn succeeds to undergo the same change. As the gastric fluid, to a certain extent, pervades the contents of the stomach, though apparently in no other way than by juxtaposition, for the arrangement of the food, above described we never found disturbed, the change in each part, which in its turn comes in contact with the stomach, is far advanced before it is in actual contact with it, and consequently is soon after this in a proper state to be moved on towards the pyloric end. Thus a continual motion is going on, that part of the food which lies next the surface of the stomach passing towards the pylorus, and the more central parts approaching the surface.

The observations and explanations offered by Philip concerning the activities of the stomach were both warmly received and rejected by his contemporaries. For instance, J. A. Paris,¹⁶ O. Halstead,¹⁵ and James Johnson¹⁷ quote the works of Philip freely and agree with his findings. On the other hand, Nathan R. Smith,¹⁸ writing three years after the publication of Philip's work, although quoting this author, attacks the entire theory of secretory functioning of the stomach as evolved by Spallanzani, Hunter and others. The vehemence of this attack by Smith is shown in the following quotation, page 25

¹⁵ Halstead, O. A Full and Accurate Account of the New Method of Curing Dyspepsia, Discovered and Practiced by O. Halstead. New York, 1830, p. 156.

¹⁶ Paris, J. A. A Treatise on Diet, Etc. New York, 1828, E. Duyckinck, Collins & Co., Collins & Hannay, and O. A. Roorbach, p. 210.

¹⁷ Johnson, James. An Essay on Indigestion, Etc. Philadelphia, 1831, Nathan Kite, p. 194.

¹⁸ Smith, Nathan R. A Physiological Essay on Digestion, New York, 1825, E. Bliss and E. White, p. 93.

It were ridiculous in us, as it has appeared in many writers on this subject, to inflict, like Falstaff, a new stab on these dead theories for the sake of making the victory, in part, our own. They are obviously inconsistent with the principles of medical logic, now well established.

Further

The sensible properties of chyme suggested to some of the older anatomists the idea that the aliment was, in the stomach, attacked by a myriad of small worms which reduced it to the uniform pulpy mass of this substance. Perhaps they were as near the truth as those who consider the process to be performed by a chemical agent, for neither the existence of these wonderful worms, nor of the less wonderful fluid can be proved, and if we reason from the effect alone, we should with more propriety ascribe it to the former.

Reaumur, Spallanzani, Hunter, Magendie, Fordyce, Prout, Philip and Paris to the contrary, one might add. However, the observations of Philip have in principle withstood adverse criticism.

DISCUSSION

From the foregoing references to the literature and from the results of a limited number of gastric analyses it appears that the gastric content is not of a uniform acid concentration and that the stomach does not act as a churn or general mixing apparatus. If such conditions are to be accepted as normal then it appears that any single sample of the gastric content at any time following the taking of a meal, of necessity, will not be representative of the remaining content. A given sample of the gastric contents can only be of value when it is definitely known from which portion of the stomach it was removed.

A correct analysis of gastric activity can only be made when it is known (1) from what portion of the stomach the tube is delivering, (2) whether or not duodenal regurgitation is occurring, (3) the degree of motility of the stomach, (4) the secretory power of the stomach and, (5) the effects of withdrawals on the motility and secretory functions. If the works of Rehfuess, Hawk, Boldyreff and others are correct, and we have no reason to discredit them at the present time, then the position of the stomach tube tip becomes of relatively little importance for the reason that a tip in the pyloric antrum may deliver not only samples of high acid concentration but also samples of low acid concentration and high tryptic value because of duodenal regurgitation. Gastric motility may also shift the tube tip about in such a manner as to cause the delivery of material from any portion of the stomach. The rapidity with which the stomach empties itself might also alter the degree of acid concentration for any set of samples withdrawn. It further appears that the response of a given stomach to a test meal should also be considered, as recent work has shown that the same stomach responds differently to different types of foodstuffs. The usual test meal, especially the one used in the present experimental work, is far

from palatable even though it allay hunger contractions. Lastly, as pointed out above, the effects of the sudden withdrawal of 10 cc amounts from the stomach at stated intervals should be considered. It is not justifiable, at least at the present time, to assume that the removal of such quantities from the stomach is in any way the equivalent to the ejection of chyme through the pyloric sphincter into the duodenum. The tonicity of the stomach, especially of the fundic end, is usually just sufficient to approximate the walls of the stomach against the mass contained. Under such a degree of tonicity the fundus presents new material to the pyloric region, small portions of which are expelled more or less rhythmically into the duodenum. Hence, it appears that a sudden reduction in the mass within the stomach would result in at least a temporary relative loss of tonicity, i.e., the stomach would have to reinstate a tonicity sufficient to exert a pressure on the gastric mass. This point seems to be of some interest although the figures given above are not sufficient to establish the explanation given.

From the above considerations it becomes evident that "correlation of all data is essential to successful interpretation of gastric curves."² Further than this, it appears that gastric curves, in a majority of cases, cannot justly be taken to represent the powers of the stomach to form acid from any point in the curve cannot be taken as indicative of the mass as a whole left in the stomach at that time of withdrawal. In the words of Cannon,⁸ who quotes Sick, Prym et al. "Evidently, if the contents are not a uniform and homogenous mixture, not only may the stomach tube give wrong testimony, but the food even when expressed as a whole be equally deceptive." When added to this we find duodenal regurgitation with its consequent reduction of the acid concentration in the pyloric portion of the stomach, the chances for "wrong testimony" become greatly increased.

In spite of the fact that the present work tends to diminish the importance of the "fractional method," it is not the desire to leave the impression that gastric analyses are of but little clinical importance. It is true that "The use of the roentgen ray and exploratory incision," as suggested by Green,¹⁹ "has displaced the systematic examination of the gastric contents to an unjustifiable degree. In relation to the stomach the tube is the most valuable of our aids to diagnosis and therapy alike."

SUMMARY AND CONCLUSION

1 The acid concentration of the gastric contents is not, in the majority of cases (nineteen young men), constant in all portions of the gastric content at the end of one hour of digestion

¹⁹ Green, C. L. *Medical Diagnosis for the Student and Practitioner*, Philadelphia, P. Blakiston's Son & Co.

2 The withdrawal of gastric contents for the purpose of determining the acid concentration, the type of the meal, the position of the tube tip, and duodenal regurgitation are factors which militate against the acceptance of "Fractional Curves" as indicative of the secretory functions of the stomach. The variations in the curves of the twenty men studied by the "fractional method" may, in part, be the result of these physiologic factors.

3 As pointed out by Gorham these physiologic factors, in great part may be held as responsible for the various "Secretory Curves" which formerly were considered functional alterations in the power of the stomach to form acid.

A CASE OF ALKAPTONURIA WITH A STUDY OF ITS METABOLISM^{*}

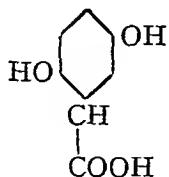
R B GIBSON, PH D AND C P HOWARD, M D
IOWA CITY

REPORT OF CASE

History—A male, aged 44 years, entered the medical clinic in May, 1920, with symptoms and signs of a midbrain lesion, possibly due to a tuberculoma. He was almost completely aphasic and no history of his family antecedents or his previous medical condition could be elicited.

Examination—The first two routine examinations of the urine revealed a slight reduction with Fehling's solution but no other abnormality was then noted. A day or two later, we were struck with the appearance of a specimen of urine because of its peculiar mahogany brown color. We were told that it belonged to our patient with the midbrain lesion and that it reduced Fehling's solution, which first appeared brownish black and later gave a reddish copper sediment. We naturally immediately suspected alkapton as the cause for the change. Our suspicions were further confirmed when it was found that the color of the freshly voided urine was the normal amber, which gradually deepened to dark mahogany brown on standing. Further special tests of the urine confirmed the presence of alkapton and the patient was transferred to the metabolism unit for more careful study.

Before giving the results of this study, let us briefly review the main points known about this anomaly of metabolism. Boedeker,¹ in 1861, first detected a reducing substance in the urine to which he gave the name "alkapton" (a bilingual word derived from alkali and katein, to absorb greedily) because of its behavior toward alkalies. In 1891, Wolkow and Baumann² identified alkapton as homogentisic acid with an empirical formula of $C_8H_8O_4$. Later, Baumann and Fraenkel,³ Osborne,⁴ and Neubauer and Flatow⁵ successfully synthesized it and proved its structural formula to be that of para-dioxyphenylacetic acid (hydroquinon-acetic acid)



This substance can readily be separated from the urine by the addition of solid neutral lead acetate, the resultant dense precipitate filtered off,

^{*} From the Medical Clinic and the Chemical Research Laboratory of the University Hospital of the State University of Iowa.

1 Boedeker, C. *Ann d Chem u Pharmakol* **117** 98, 1861.

2 Wolkow, M., and Baumann, E. *Ztschr f physiol Chem* **15** 228, 1891.

3 Baumann, E., and Fraenkel, S. *Ztschr f physiol Chem* **20** 219, 1895.

4 Osborne, W. A. *J Physiol* **29** 13, 1903.

5 Neubauer, O. and Flatow, L. *Ztschr f physiol Chem* **52** 375, 1907.

and the clear yellow filtrate allowed to stand in the cold when the crystals of lead homogentisate appear, which later can be purified into colorless crystals of homogentisic acid with a melting point of 146 C

Qualitative Tests—The urine on exposure to the air or on the addition of alkali turns brown or even black. It reduces copper solutions but does not yield to the fermentation test, does not reduce alkaline bismuth solutions and does not rotate polarized light. An ammoniacal solution of silver nitrate is quickly reduced by it in the cold. Lastly, when a dilute solution of ferric chlorid is added to the urine, drop by drop, a deep blue color appears for a moment, as each drop falls, until oxidation is complete.

Incidence—It is a rare condition. Not more than seventy cases exist in the literature and only eleven have been reported from America. Barton Brune⁶ of Baltimore, in 1886, reported the first case in this country. Subsequently, cases were published by Marshall⁷ of Philadelphia, Ogden⁸ of Milwaukee, and Osler⁹ of Baltimore, etc., making a total of twelve, with ours. There is an unquestionable familial tendency, especially in the offspring of consanguineous marriages, according to A. E. Garrod¹⁰. Though familial, it is not congenital, and no reports of parent and child being afflicted have ever appeared. Garrod's series of eleven cases occurred in four families in all of which the parents were first cousins. In its incidence, it shows a striking similarity to albinism. It is more common in males than females.

Symptomatology—As a rule, there is no inconvenience experienced by the patient, except, possibly, the staining of the underclothes by the urine. Occasionally, there is dysuria. It is often not recognized until the patient submits to a life insurance examination and the applicant is rejected as a diabetic. One of Osler's patients had been treated for mild glycosuria by four prominent continental physicians, before consulting Osler. A more careful analysis of the urine by T. B. Futcher¹¹ revealed the fact that the reducing substance was alkapton. Though usually constant, it may be intermittent, and absent for years at a time. A small proportion of the cases (twenty-four cases up to 1919) are associated with ochronosis or blackening of the cartilages and fibrous tissues and pigmentation of the skin. Alkaptonurics, with an associated ochronosis, may reveal during life pain, swelling and deformity of one or more of the larger joints of the extremities or of the small joints of the hands—a condition termed by Osler ochronotic arthritis. To

6 Brune, B. Boston M. & S. J. **115** 621, 1886, **116** 83, 1886

7 Marshall, J. Med. News, **50** 35, 1887

8 Ogden, H. V. Ztschr. f. Physiol. Chem. **20** 281, 1895

9 Osler, W. Lancet, **1** 10, 1904

10 Garrod, A. E. Inborn Errors of Metabolism 1909, London, p. 41

11 Futcher, T. B. New York M. J. **67** 67, 1898

explain this condition, Albrecht¹² considered that homogentisic acid, or one of its derivatives, combined with the chondromucoid or the chondroitin sulphuric acid of the cartilage to form the pigment

In a few rare instances temporary alkaptonuria has been found in association with gastro-enteritis, gastric ulcer, pulmonary tuberculosis, peritoneal tuberculosis and diabetes. Falta and Langstein¹³ have shown that some patients with grave diabetes have less power of destroying homogentisic acid administered by mouth than have normal persons

Pathogenesis—It is a freak of metabolism, or as Garrod so well expresses it, "an inborn error of metabolism." The error consists in a failure to complete the catabolism of the aromatic fractions of the

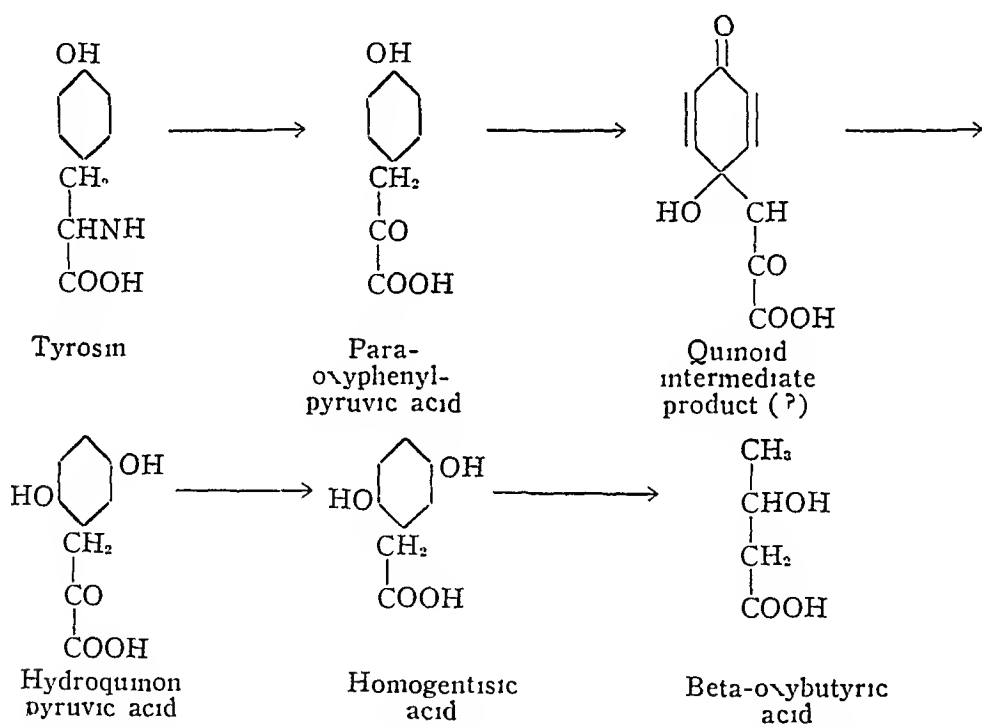


Fig 1—Scheme of the origin of homogentisic acid (adapted from Neubauer)

proteins, tyrosin and phenylalanin. The tyrosin and phenylalanin of both the food and tissue proteins are concerned. During fasting the output of homogentisic acid is diminished, but is not arrested. A diet rich in proteins greatly increase the output, and when tyrosin or phenylalanin is given by mouth to an alkaptonuric an almost corresponding quantity of homogentisic acid appears in the urine. On the other hand, these amino-acids, when taken by normal persons, are destroyed and the benzene ring broken up. The quantities of homogentisic acid excreted by different alkaptonurics are singularly uniform when the nature of

¹² Albrecht, H. *Ztschr f Heilk* **23** 366, 1902

¹³ Falta, W., and Langstein, L. *Ztschr f physiol Chem* **38** 513, 1903

the diet and ages of the patients are allowed for. This amount varies from 3 to 6 gm in twenty-four hours, though rarely 16 gm and even 18 gm have been found. Garrod states that there is reason to believe that the error is in all instances complete and maximal in degree. Wolkow and Baumann² suggested that the change from tyrosin to alkapton might take place in the intestine under the influence of a rare specific microbe, but Garrod supports the view that the change occurs in the tissues after the absorption of the tyrosin rather than in the alimentary canal. Normally at least a part of the benzene ring may be broken up in the body, involving the production of beta-oxybutyric acid, two of the carbon atoms of the latter are derived from the phenyl ring and two from the side chain, according to Wakeman and Dakin¹⁴ (Fig 1), neither tyrosin nor phenylalanin yield glucose in the phloridzin diabetic dog as do some of the non-ring-form amino-acids (Lusk)¹⁵

TABLE 1—DETERMINATION OF HOMOGENTISIC ACID

Day	Total N, Gm	Homogentisic Acid	
		Gm	H-N Quot
6/11/20	6.27	3.77	60
6/12/20	7.84	4.78	61
6/13/20*	6.08	6.27	103

* Two grams of tyrosin ingested

In alkaptonurics, the conversion of tyrosin and phenylalanin into homogentisic acid is so complete that the ratio of homogentisic acid to the total nitrogen in the urine tends to be constant and the same for all cases (Garrod). The homogentisic acid amounts to 40 or 45 per cent of the total nitrogen figure.

Gross,¹⁶ in 1914, found that the serum of normal animals and man destroys homogentisic acid, probably producing acetone, this change is brought about by an active enzyme which seems to be absent in the alkaptonuric. Since our own observations have been made, Katsch¹⁷ has reported the case of a boy in whom he had earlier observed the disappearance of homogentisic acid from the urine during a starvation period. Subsequent study proved that the diminution of the alkapton condition was associated with ketone acidosis, at least the acidosis induced by a diet poor in carbohydrates reduced the H/N quotient to 1.5. The coincident acetone elimination was greater than theoretically could be obtained from the aromatic amino-acids alone.

14 Wakeman, A. J., and Dakin, H. D. *J. Biol. Chem.* **9** 139, 1911.

15 Lusk, G. *The Science of Nutrition*, Ed. 3, W. B. Saunders Co., Philadelphia, 1917, p. 196.

16 Gross, O. *Biochem. Ztschr.* **61** 165, 1914.

17 Katsch, G. *Deutsch. Arch. f. klin. Med.* **127** 210, 1918, **134** 59, 1920.

TABLE 2—A NITROGEN PARTITION AND HOMOGENTISIC ACID EXCRETION

Day	Volume, C c	Specific Gravity	Total N, Gm	Homogen- istic Acid		Urea N		Ammonia N		Uric Acid N		Creatinin N		Creatin N		Undetermined N	
				Gm	H/N	Gm	%	Gm	%	Gm	%	Gm	%	Gm	%	Gm	%
6/18	1,040	1.030	11.76	6.08	51			0.952	7.36	0.229*	1.77	0.423	3.60				
6/19	2,000	1.025	12.93	5.77	45	8.40	65.0	0.838	7.21	0.271	2.33	0.398	3.03				
6/20	1,250	1.023	11.62	6.70	57	6.51	56.0	1.120	7.46	0.233	1.55	0.448	3.86	0.085	0.56	4.68	31.2
6/22	2,000	1.018	15.00	6.27	42	8.46	56.4			0.189	1.35	0.435	2.90	0.064	0.46	4.77	34.4
6/23	1,340	1.032	13.86	7.58	55	7.24	52.2	1.160	8.35			0.438	3.16				

B Sulphur Partition									
Day	Total SO ₃		Inorganic S SO ₃ SO ₃		Etheral Sulphur SO ₃		Neutral Sulphur SO ₃		
	Gm	%	Gm	%	Gm	%	Gm	%	
6/22	2.356	81.47	1.919	81.47	0.140	5.94	0.297	12.59	
6/23	3.218	78.33	2.520	78.33	0.319	9.90	0.379	11.77	

* By Morris' method (J Biol Chem 37 231, 1919), other determinations of uric acid are according to Folin and Wu

In order to prove metabolically the present case one of alkaptonuria, the patient was put on a constant diet (70 gm protein, 83 gm fat, and 253 gm carbohydrate, no meat or soup), the homogentisic acid and the total nitrogen excretion were determined, and the effects of tyrosin added to the diet observed. Homogentisic acid was determined by Garrod's modification of Baumann's method.

The homogentisic acid is higher in relation to the total nitrogen than is stated by Garrod, owing to the fact that milk was a principal constituent of the diet, and casein contains more of the aromatic amino-acids than do many of the common proteins. The characteristic increase in homogentisic acid is observed to follow the tyrosin ingestion. Subsequent determinations (Table 2) show a quotient of 42 to 57, with an average for the five days of 50, at this time the patient was on a varying diet and the food intake was not controlled.

The distribution of the several nitrogenous constituents of the urine in alkaptonuria is said to remain normal (Lusk)¹⁵. Only by high elimination of the homogentisic acid is the ammonia increased (Schumm)¹⁸. Adler¹⁹ states that purine metabolism is not disturbed. Ravold and Warren²⁰ found the urea below the average and the uric acid somewhat diminished. Interest in the sulphur metabolism was early directed to the possible conjugation of the homogentisic acid, but the acid was found to be free or combined as simple salts (Meyer)²¹.

Certain difficulties are encountered in the determination of uric acid and creatinin by current methods in the presence of homogentisic acid. This is especially true of creatinin, which was finally determined after the removal of homogentisic acid with lead acetate. The silver lactate reagent of Folin and Wu²² was promptly reduced in the cold, a reaction which should serve to identify homogentisic acid as other reducing substances that are found in the urine do not behave in this way.

In our case we found a high ammonia nitrogen, low urea nitrogen, moderately high uric acid nitrogen figures, creatinuria, and high undetermined nitrogen. The sulphur excretion seems to have been essentially normal.

18 Schumm, O. *Muench med Wchnschr* **51** 1599, 1904.

19 Adler, O. *Biochem Ztschr* **21** 5, 1909.

20 Ravold, A., and Warren, W. H. *J Biol Chem* **7** 465, 1910.

21 Meyer, E. *Deutsch Arch f klin Med* **70** 443, 1901.

22 Folin, O., and Wu, H. *J Biol Chem* **38** 459, 1919.

THE INFLUENCE OF INORGANIC IRON ON THE REGENERATION OF BLOOD AFTER HEMORRHAGIC ANEMIA *

JOHN H. MUSSER, JR., M.D.

PHILADELPHIA

The controversy over the therapeutic effect of inorganic iron has raged for several centuries, in fact, very considerably has it occupied the minds of medical men since Menghis, in 1746, reported that he had found iron in the blood of man. This controversy continued up until the past fifteen years, when Abderhalden, in 1906, abandoned his contention that iron could not be converted into hemoglobin. Since, then, it has been very generally accepted that inorganic iron is converted into hemoglobin as is the iron in the food and the so called organic iron. A re-opening of this controversy might be said to have occurred last year, when Whipple¹ and his co-workers published their studies on the regeneration of the blood after anemia. In one of this series of papers they show that ordinary inorganic iron in the form of Bland's pills has little or no effect on blood regeneration without adequate diets, and they are led to draw the conclusion that "we may not assume, without positive proof, that inorganic iron is of value in the treatment of simple anemia."

The present series of experiments were undertaken for the purpose of determining whether this conclusion would hold true in the type of anemia that represents more closely the type that is seen in clinical medicine than that which occurs after a single large or massive hemorrhage. The opportunity was presented of securing some animals which had repeatedly been deprived of small amounts of blood over various intervals of time, and which had thus been rendered anemic. The anemia that these animals showed represented very truly the type of anemia which occurs after recurring loss of small amounts of blood and which the physician is called on most frequently to treat. These animals had been bled for varied lengths of time over periods ranging from six to eight weeks, and then immediately before starting the use of iron, they were rendered more anemic by one or more large bleedings. It is, of course, recognized that the factor of damaged blood formation (which is more or less of a factor in many clinical anemias) was not included in either these or Whipple's experiments on the efficacy of inorganic iron.

* From the Department of Research Medicine, University of Pennsylvania.

1 Whipple, G. H., Hooper, C. W., and Robschert, F. S. Blood Regeneration following Simple Anemia, *Am. J. Physiol.* **53** 263, 1920.

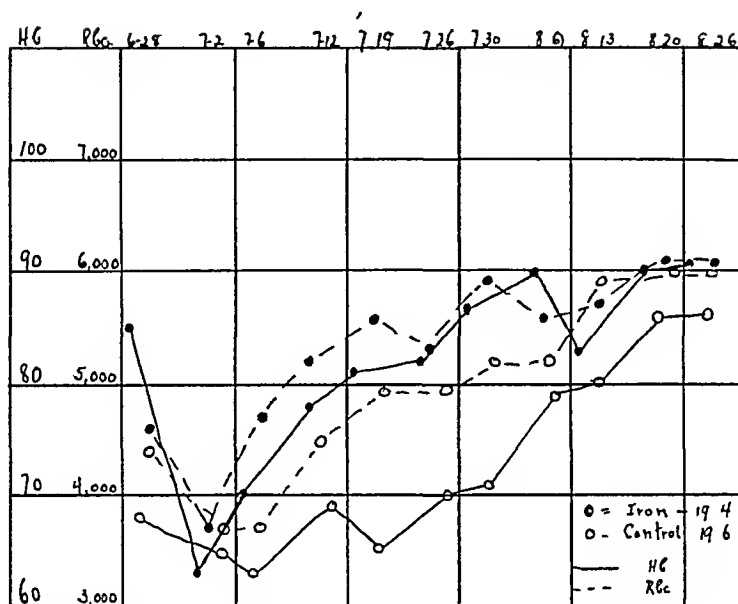


Fig 1—Hemoglobin and erythrocytes of Dog 19-4 and control

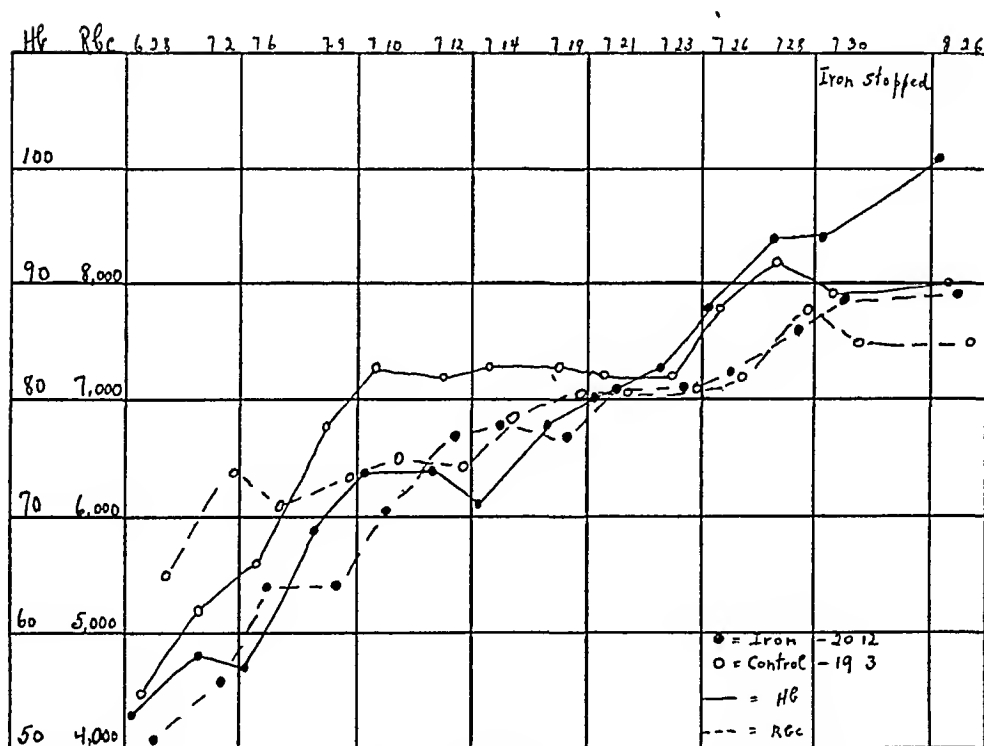


Fig 2—Hemoglobin and erythrocytes of Dog 20-12 and control

METHODS

In order to follow as carefully as possible the response of the animal to repeated hemorrhage, the following studies were undertaken hemoglobin estimation, red cell count, enumeration of the white blood cells, differential count, resistance of the red cells to hypotonic salt solution, occasional observation on the percentage of skein cells and the determination of the total blood volume. The hemoglobin was estimated by the method of Newcomer, the red cells and white cells were counted by the usual methods on the Levy counting chamber, the resistance of the red cells was determined by the addition of a known quantity of red cells to a known quantity of hypotonic salt solution in ascending proportions, ranging from 0.25 to 0.5 per cent, the skein cells were stained vitally with brilliant cresyl blue in potassium oxalate and counted with the oil immersion lens, and the blood volume was estimated by the method of Keith, Rountree and Geraghty. The blood for counting was collected from a puncture in the ear vein, made at approximately the same time each day and always before the animal had been fed. The blood for the volume estimation was taken from the jugular vein. The bleeding of the animals was from the jugular vein and in various quantities. The animals selected were apparently healthy, young adult dogs, and in order that the factor of diet might more closely approach that of the normal and bear no relationship to anemia, they were fed on the standard diet for all the dogs in the kennels, which consisted largely of hospital scraps. The animals were healthy throughout the course of the experiment and most of them gained weight. The blood counts were made at the start of the experiment and subsequently every second or third day. Except in one experiment, the dog which was given the iron and the control dog were studied the same day.

The iron was given in the form of equal parts of ferrous sulphate and sodium bicarbonate in capsule. This combination of iron presents the iron carbonate in a soft and powdery form. The capsule can readily be placed in the dog's mouth and pushed down with the finger until the dog swallows it. The gelatin capsule dissolves rapidly, and in such a form that it would not pass through the intestines undissolved as do the tightly compressed Bland tablets at times. The dose of iron given each dog was estimated on the basis that an average dose for a human being of 65 kg weight is 1 gm a day. A proportionate dose was worked out for each dog according to his body weight on commencing the experiment. In order to make sure that sufficient iron was given, this dose was doubled, that is to say, an amount of iron equivalent to 2 gm per diem for man, was given each dog.

EXPERIMENTAL DATA

Experiment 1—In the first experiment, two dogs were employed which had been a long time splenectomized and which had been rendered anemic as the

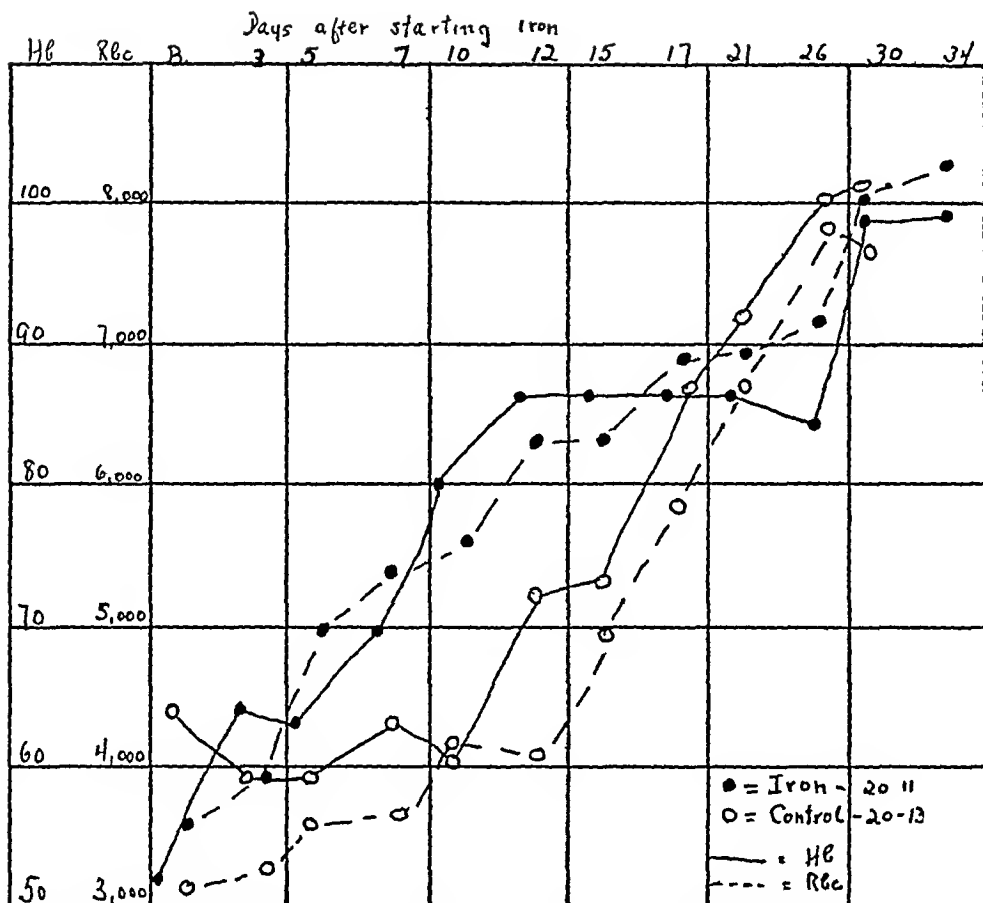


Fig 3—Hemoglobin and erythrocytes of Dog 20-11 and control

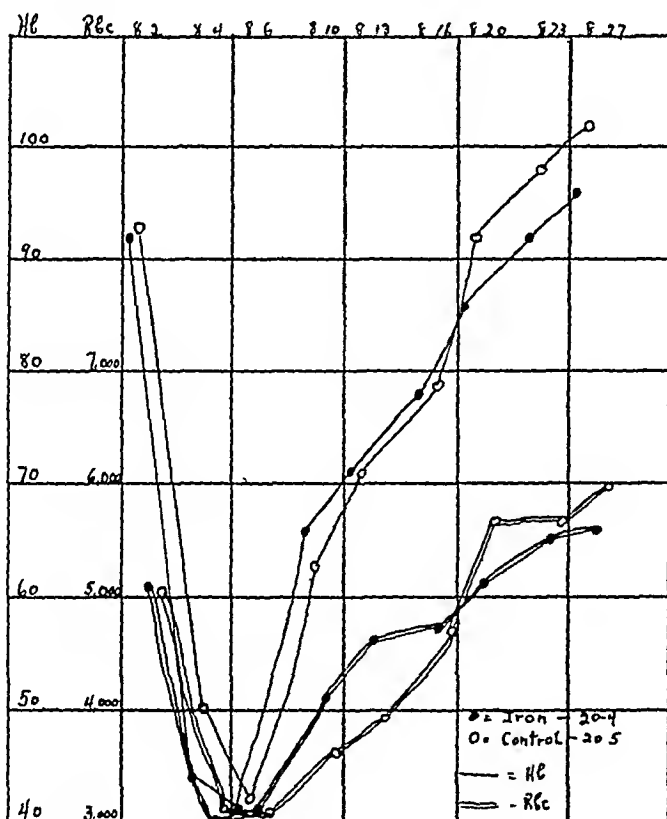


Fig 4—Hemoglobin and erythrocytes of Dog 20-4 and control

result of weekly bleedings of 100 cc for a period of two and one-half months. Following the preliminary study, the two animals were bled respectively 400 cc. The amount of blood taken from both animals was approximately the same. During a period of nearly two months blood counts were made, as a rule, every second or third day with occasionally three days intervening between counts. During this interval of time it can be noted that the dog which was given iron following the preliminary hemorrhage, 19-4, returned more rapidly than did the control dog, 19-6, to a higher hemoglobin estimation and, likewise the

TABLE 1—DOG 19-4 LONG TIME SPLENECTOMIZED BLED 100 CC A WEEK FOR 2½ MONTHS CONTROLLED BY 19-6

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
6/28	85	4,640	26-38	0-400		13,450
6/30	Bled, 430 cc	3,520	26-38			
7/ 1	Start Iron					
7/ 2	63	3,770			T V 950 T P 740 T R 210	13,250
7/ 4	68	4,040				
7/ 6	70	4,770	26-38	4-100		13,240
7/ 8	72	4,970				
7/10	74	4,820				
7/12	78	5,240	28-38	2-100	T V 1,800 T P 757 T R 543	13,200
7/14	83	5,810				
7/16	83	5,670	26-38			
7/19	81	5,680				
7/21	78	5,470	28-40	1-200		
7/23	78	4,750			T V 1,042 T P 687 T R 355	
7/26	82	5,360				
7/27	86	5,160				
7/30	87	5,910	30-44			
8/ 2	90	5,630			T V 1,400 T P 895 T R 505	
8/ 6	79	5,670				
8/10	79	5,890	30-40		T V 1,140 T P 752 T R 388	13,260
8/13	83	5,720				
8/16	86	5,650	28-40	1-200		
8/20	90	6,130			T V 1,220 T P 750 T R 464	
8/24	95	6,040	26-40	1-500		
8/26	91	6,010			T V 1,175 T P 753 T R 422	13,370

number of red cells was increased more rapidly (Tables 1 and 2). However, dog 19-6, the control animal, started with a decidedly lower hemoglobin percentage than did dog 19-4, while in the course of the experiment both animals arrived about the same time at the high water mark in their counts. The resistance of both animals to hypotonic salt solution was about the same and varied somewhat from day to day. The marrow response, as shown by the skein cells, was somewhat more pronounced in the dog which was receiving the iron. The blood volume showed in the dog which was the more anemic, the greater reduction at the start of the experiment, and this continued about the same through the length of the experiment. The last volume estimation, however, did show, in spite of the fact that the volume of 19-4 was higher than 19-6, that the total red cells of this latter dog were considerably higher. During

the course of the experiment it is to be noted that dog 19-4, which was taking the iron, developed an acute drop in the number of red cells and percentage of hemoglobin, as well as in the total blood volume, without any demonstrable cause. From this causeless short period of increased anemia the animal rapidly returned to its former standard.

Experiment 2—The second experiment was analagous to the first, except that the dogs employed had not been splenectomized. Dog 20-12 had been bled steadily for a period of eight weeks, in quantities of about 150 cc every week.

TABLE 2—DOG 19-6 LONG TIME SPLENECTOMIZED BLED 100 CC A WEEK FOR 2½ MONTHS CONTROL OF 19-4

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
6/28	68	4,450	28-40	0-400		14,000
6/30	Bled, 400 cc	3,640	26-38		T V 845	
7/2		3,750			T P 660	13,880
					T R 185	
7/4	65	3,560				
7/6	63	3,790	26-40	5-100		13,050
7/8	59	4,160				
7/10	61	4,430				
7/12	69	4,560	24-38	1-500		
7/14	66				T V 1,085	
7/16	64	4,890	28-36		T P 756	13,430
					T R 279	
7/19	65	4,970				
7/21	65	4,800				
7/23	62	4,990	26-38	1-400	T V 970	13,600
7/26	70	4,960			T P 650	
					T R 320	
7/27	71	5,060				
7/30	71	5,260	26-42		T V 1,150	
8/2	79	5,290			T P 758	
					T R 392	
8/10	76	5,870	26-38	1-400	T V 1,158	13,130
					T P 730	
					T R 428	
8/13	80	5,940				
8/16	83	6,210	24-38	1-400	T V 1,030	
					T P 628	
					T R 402	
8/20	86	6,070				
8/24	92	6,160	24-38	1-500	T V 1,070	13,300
					T P 643	
					T R 527	
8/26	86	6,080				

The control dog had been bled almost steadily for seven months. At the beginning of the experiment, the two dogs had relatively the same hemoglobin percentage. Dog 20-12, however, had a red cell count of a million and a half less than the control dog, 19-3 (Tables 3 and 4). The control dog was also the much larger animal, with red cells which were more resistant than in the other animal. The blood volume of the two dogs was about the same on commencing. However, the control dog's subsequent estimation showed a much larger total volume. While the red cells were absolutely greater in number in this animal than in his fellow, the much larger plasma content would indicate a relatively lessened concentration of the erythrocytes in the peripheral circulation as well as a lower hemoglobin estimation, so that in this animal the blood volume estimation shows that the control dog is regenerating more rapidly and more

TABLE 3—DOG 20-12 BLED 150 c c WEEKLY FOR 8 WEEKS, LAST BLEEDING 6-28 CONTROLLED BY 19-3

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
6/28	53	4,016	30-42	10-300		9,510
7/ 1	Start iron				T V 967	9,950
7/ 2		4,650			T P 725	
					T R 242	
7/ 6	57	5,400	30-44	2-100		11,000
7/ 8	69	5,440				
7/10	74	6,080				
7/12	74	6,760	28-46	1-200		
7/14	71	6,850				
7/16	78	6,980	30-44		T V 850	
					T P 552	10,360
					T R 298	
7/19	75	6,880				
7/21	80	7,170				
7/23	83	7,100	30-44		T V 796	
7/26	88	7,290			T P 478	
					T R 318	11,400
7/28	84	7,650	28-44			
7/30	84	7,990			T V 1,040	
	stop iron				T P 655	
					T R 385	
8/26	101	7,970			T V 1,235	
					T P 655	11,200
					T R 580	

TABLE 4—DOG 19-3 BLED 100-150 c c WEEKLY FOR 7 MONTHS, LAST BLEEDING 6-28 CONTROL OF 20-12

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
6/28	55 5	5,500	26-36	0		15,730
7/ 2	62	6,420			T V 995	15,560
					T P 735	
					T R 260	15,440
7/ 6	66	6,080	26-38	1-200		
7/ 8	78	6,380				
7/10	83	6,570				
7/12	82 5	6,440	24-38	1-200		
7/14	83	6,880	26-38			
7/19	83	7,040				15,660
7/21	81	7,090				
7/23	82	7,130	24-36			
7/26	88	7,270			T V 1,234	
					T P 740	
					T R 494	
7/28	92	7,880				14,790
7/30	89	7,530	24-40		T V 1,394	
					T P 850	
					T R 544	
8/26	90	7,520			T V 1,332	
					T P 800	
					T R 532	15,140

TABLE 5—Dog 20-11 BLED 900 CC IN SMALL BLEEDINGS, IN TWO MONTHS THEN 100 CC EVERY OTHER DAY FOR THREE DAYS, FOLLOWED BY A BLEEDING OF 350 CC CONTROLLED BY 20-13

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
7/16	52	3,540	26-40	14-100		17,330
7/19	Start Iron 64	3,970			T V 1,142	16,040
					T P 812	
					T R 330	
7/21	63	5,080	28-42	10-100		16,140
7/23	70	5,490	28-42	6-100		
7/26	80	5,650	26-42	3-100		
7/28	86	6,320			T V 1,320	
					T P 885	
					T R 435	
8/ 2	86	6,360	26-42	4-100		17,690
8/ 4	86	6,920			T V 1,340	
					T P 856	
8/ 8	86	6,950	26-42	1-300	T R 484	17,910
					T V 1,440	
					T P 861	
8/13	84	7,190			T R 576	
					T V 1,398	
					T P 740	
8/17	98	8,040	28-42	2-500		
8/20	98	8,350			T V 1,398	
					T P 740	
					T R 058	

TABLE 6—Dog 20-13 BLED 600 CC IN SMALL BLEEDINGS IN 6 WEEKS THEN DAILY BLEEDING OF 100 CC FOR THREE DAYS AND 250 CC ON EACH OF THE FOLLOWING THREE DAYS CONTROL OF 20-11

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
7/26	64	3,120	24-40		T V 813 T P 502	15,600
					T R 311	
7/28	59	3,280	26-40	5-100	T V 1,026	15,510
7/30	59	3,600	28-42		T P 797	
					T R 223	
8/ 2	68	3,630	28-42		T V 1,225	15,550
8/ 4	60	4,120			T P 857	
					T R 368	
8/ 8	72	4,010	28-42	1-400	T V 1,270	15,550
8/13	73	4,970	26-40	1-600	T P 825	
					T R 445	
8/17	86	5,860	28-38	4-400	T V 1,200 T P 722	
					T R 478	
8/20	92	6,740	28-40	1-400	T V 1,560	15,810
8/23	100	7,800			T P 845	
8/26	101	7,650			T R 715	

completely than the simple peripheral counts would indicate. The control dog, in the month that the counts were being made, recovered from his anemia rather rapidly, as did the other dog. At the end of the month, the hemoglobin of the control dog was slightly higher, while the red cells were slightly lower, than the other animal. The blood volume of the larger dog, that is the control, had increased, as was to be expected, very much more rapidly than the other animal, and the same holds good in the total number of red cells. At the end of the month the iron was stopped. Nothing further was done to the dogs until a month later, when they were again counted. The count at this time showed that the dog which had been receiving the iron, had increased its hemoglobin percentage to 101, and apparently had not recovered from its anemia when the

TABLE 7—DOG 20-4 LONG TIME SPLENECTOMIZED CONTROLLED BY 20-5

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
8/ 2	92	5,190				10,000
8/ 3	Bled, 350 c c					
8/ 4	44	2,980	26-40			10,100
	Bled, 175 c c					
	start iron					
8/ 6	41	3,010	28-42	1-100	T V 895	
8/10	66	4,040	28-40	4-100	T P 597	
					T R 298	
8/13	71	4,620				11,500
8/16	78	4,780	28-42	2-100	T V 940	
8/20	86	5,110			T P 563	
					T R 377	
8/23	92	5,520	28-42	1-100		12,400
8/27	96	5,680				

TABLE 8—DOG 20-5 LONG TIME SPLENECTOMIZED CONTROL OF 20-4

Date	Hb	R B C	Resistance	Sk	Blood Volume	Weight
8/ 2	93.5	5,120				16,000
8/ 3	Bled, 425 c c					
8/ 4	50	3,070	30-42			16,600
	Bled, 350 c c					
8/ 6	42	3,100	32-44			16,800
8/10	63	3,650	28-42	3-100	T V 1,065	16,730
					T P 756	
					T R 309	
8/13	71	3,910				
8/16	79	4,750	28-42	4-300		16,800
8/20	92	5,740				
8/23	98	5,690	28-40	2-300	T V 1,320	16,900
					T P 738	
					T R 582	
8/27	102	5,980				

count had been stopped a month earlier, whereas, with the control dog, figures that were normal for that dog had been reached when the experiment was stopped. It is interesting to note that throughout the course of this experiment the resistance of the control dog was always greater than the animal which was receiving the iron.

Experiment 3—The procedures in this experiment were altered somewhat. Two animals, 20-11 and 20-13, which had been bled small quantities every six or eight weeks, were then bled 100 c c for three consecutive days, and then larger bleedings for the next three days. When these bleedings were stopped, the dog which was to receive the iron, 20-11, had a lower hemoglobin estimation and a higher red cell count than did the control dog (Tables 5 and 6). This dog

also showed a very vigorous marrow response, as shown by the skein cells. The total blood volume was considerably higher than with the control. The severity of the anemia is shown by the blood volume estimations to be much greater than the peripheral blood counts would indicate. Regeneration of the red cells after the hemorrhage was more rapid in the dog taking the iron. On the other hand, in the control dog, the hemoglobin rose slowly but ultimately attained higher figures than it did with the iron-taking animal. In this experiment the resistance of the red cells to hypotonic salt solution was slightly greater in the control animal. The skein cells in the animal receiving the iron were more frequent during the course of the experiments than in the animal that was used as a control. To return again to the blood volume, it can be seen that the total volume of the iron taking dog, the heavier of the two, remained greater than in the control dog until the last count. Likewise, the total reds of the control dog were considerably higher at the end of the experiment than in the test dog. Both animals increased in weight during the course of the experiment.

Experiment 4—The last experiment was undertaken for the purpose of determining the rapidity of the blood regeneration of two splenectomized animals who had been splenectomized a long time. Both animals, 20-4, and 20-5, were relatively anemic at the start of the experiment (Tables 7 and 8). Each animal was bled as large a quantity as could be taken from him on two successive days and both were rendered extremely anemic. The anemia of the control dog, the smaller of the two, was greater than that of the other after the hemorrhages. Both dogs returned, in a period of a little over three weeks, to a higher standard than before the bleeding. The resistance of the red cells in the two animals during the course of the experiment was about the same. The skein cells showed a somewhat more active marrow response in the iron taking animal. In this latter dog, the blood volume was also greater as were the total reds. Both dogs gained weight.

DISCUSSION

The results of these experiments would seem to substantiate the contention of Whipple that under ordinary circumstances the giving of inorganic iron does not have a particularly marked effect on blood regeneration and the correction of hemorrhagic anemia in experimental dogs. It may be contended, and, we believe, rightly so, that the return to the normal in these animals is so rapid that the iron can have but little effect when nature is taking care of the anemia so thoroughly. However, in two of the dogs, which had been rendered anemic by repeated bleedings and by a long time splenectomy, the return to normal was relatively slow, yet in neither the control, 19-6, or the experimental dog 19-4 was there any obvious or material difference in the capacity of the regeneration of the red cells or hemoglobin. Difference, of course, exists between the dog taking iron and the controls, but only such as would be expected. It would be almost impossible to obtain animals which were alike in every respect. The hematopoietic systems of no two animals will respond in exactly the same manner. In considering experimental work of this type, various factors must be taken into consideration. The resistance of the red cells to hypotonic salt solution in some animals is greater than in others, and this must play some factor in altering the course of anemia. In

some animals, the response of the marrow and the stimulation of hemorrhage is greater than in other animals. This can be demonstrated by the spleen cells. In some animals the blood volume is greater and better sustained than in others. Lastly, there is no absolute mean normal count as these and other factors produce differences in the hemoglobin and red cells count in the different dogs.

SUMMARY

After hemorrhagic anemia in otherwise normal dogs, under standard conditions, dogs receiving iron in two of four experiments (Experiments 1 and 3), regenerated blood more rapidly than their controls during the early stages but both test and control dogs attained figures approximately normal in about the same time interval. In the other two studies, regeneration is seen to be more rapid and complete in the control dogs, particularly when the blood counts are analyzed in relation to the blood volume changes.

CONCLUSION

The administration of inorganic iron cannot be said to produce any constant alteration in the course of an experimental hemorrhagic anemia.

A NEW METHOD OF INTERPRETATION OF THE RENAL FUNCTION TEST MEAL*

HERMAN SHARLIT, M D

AND

WILLIAM G LYLE, M D

NEW YORK CITY

In a previous paper ¹ we discussed the renal functional test meal, the interpretation of renal function as judged by specific gravity variation of the two hour specimens and character of night urine, and offered clinical evidence purporting to illustrate the influence of several extrarenal factors ² on the test meal reaction. These extrarenal factors interfere considerably with the uniformity of response to the test meal by the same individual, at least upon viewing the responses in the manner heretofore suggested by Mosenthal. Subsequent to the publication of our study, Mosenthal ³ reported further studies in renal response to diets in which he felt obliged to revise his previous dicta as to evidences of normality and the significance of certain deviations therefrom, although still adhering to volume and specific gravity as the essential criteria of renal function. The nature and direction of his revision can be gleaned from an inspection of Table 1, which contains in parallel columns his earlier and later standards of normality.

The studies on which this report is based were completed previous to the publication of Mosenthal's later investigation, and were the outcome of an effort to circumvent the difficulties presented by his earlier definitions on interpretation of the test diet, and become, it appears to us, more effective in the light of his later report. We reserve for another time, however, the presentation of studies based on the added data supplied us in his more recent publication.

We felt satisfied that extrarenal influences on the reactions to the renal functional test diet operated chiefly through their effects on the supply of fluid to the kidney. We decided, therefore, to study the reactions chiefly in terms of the total solids eliminated, and some of the results of such studies we desire to make the subject of this report.

*From Pathological Laboratory of Roosevelt Hospital and the Harriman Research Laboratory.

1 Lyle, W G and Sharlit, H. Arch Int Med **21** 366 (March) 1918.

2 By the phrase "extrarenal factors" in connection with reactions to the test diet, we refer to those influences upon renal excretion that occur independent of the stimulation supplied the kidney by the test diet and hence a variable influence not controlled by the test.

3 Mosenthal, H O. Arch Int Med **22** 770 (Dec) 1918.

The test meals used followed that outlined by Mosenthal⁴ All the meals were served from a special diet kitchen conducted under the supervision of the pathological laboratory at the Roosevelt Hospital The meals were served at 8 a m, 12 n and 5 p m Two hour urine specimens were collected from 8 a m to 8 p m, and the night specimen from 8 p m to 8 a m of the following day Special care was taken to see that exactly 3 hours elapsed between the taking of the evening meal and the beginning of the collection of the night specimen

It being, of course, rather impracticable to seek to determine the total urinary solids by any analytical method, we adopted the very simple yet fairly accurate method of calculating the total solids present from the specific gravity of the specimen For accurate estimations it is quite essential that all the solids in the urinary specimens be in solution To insure such solution, we followed the procedure of warming the specimen to about 40 C at which temperature quite

TABLE 1—NORMAL STANDARDS FOR TEST MEAL FOR RENAL FUNCTION

	Earlier Standard *	Later Standard †		
		High Diet	Low Diet	Normal Diet
Maximum specific gravity	1 018+	1 018+	1 020+	1 020+
Degree variation of sp gr usually	9+	9+	9+	No value
Sp gr of night urine	1 018+	Of no significance		
Volume c c of night urine	400 c c or less	750 c c or less		
N and NaCl per cent in night urine or highest per cent in any specimen	1	Normal if 1 per cent or higher, not necessarily abnormal if less		

* Earlier standard Mosenthal, H O, and Lewis, D S Jour A M A, 1916, 67 933

† Later standard (see footnote 3)

all the urinary sediments readily went into solution with no danger of decomposition of nitrogenous solids After allowing specimens to cool down to about 28 C hydrometric readings were taken The product of the last two figures of the specific gravity and the volume in cubic centimeters divided by 1,000 and the quotient multiplied by the constant 2.3 as determined by Haeser or 2.6 as estimated by Long⁵ gives approximately the grams of solids present in the given volume of urine Being more concerned with ratios than with absolute quantities we have dispensed with the constant multiplier and so the figures used throughout this report as representing the solid content of specimens are the products of the last two figures of the specific gravity and volume in c c of the specimens and should be multiplied by the constant to give grams of solids

In the study of the test meal reaction we have concerned ourselves with several features of the eliminated urinary solids

4 Mosenthal, H O Arch Int Med 16 733 (Dec) 1915

5 Hawk's Practical Physiological Chemistry, 1913, Philadelphia, P Blakiston's Sons Co, p 434

Firstly The total twenty-four hour solids eliminated, this in the average individual without definite renal insufficiency has under the stimulus of the renal test diet approximated 25⁶

With definite renal insufficiency there have appeared decided reductions in the quantity of urinary solids for the twenty-four hour period Although this has been the general tendency, the total daily output for many individuals of the normal group has fallen well within the limits of the nephritic group and the variations for the same individual have in many instances been quite marked This was to be expected considering the many factors that enter into the determination of the total twenty-four hours urinary solids for any particular day, i e, the nitrogen salt and water balance of the body on the day of the test, the degree of stimulating power in the test diet for the particular kidneys tested, the perfect functioning of the gastro-intestinal tract assuring the passage of the stimulatory food to the blood stream, sufficient supply of water for the elimination of the solids and the several secondary factors that in turn affect such a supply The item of the total solid output for the twenty-four hours has, therefore, little significance as an index of renal efficiency except in cases where it deviates very considerably from the normal

Secondly The ratio of the quantity of solids eliminated from 8 a m to 8 p m to that eliminated from 8 p m to 8 a m the following morning, the ratio of the day to the night output of urinary solids This ratio we have designated Factor 1

In view of the fact that early renal involvement very often manifests itself as a delay in the excretion of metabolites, it is quite conceivable that such a ratio might disclose such a delay long before the excretory function has been impaired to the point of actual retention of metabolites Furthermore, a separation of the renal stimulatory period—the period of food intake and greatest activity of the integral organism, from the nonstimulatory period—the period of minimal body activity, best serves to restrict the renal activity of the latter period to responses to stimulation by the circulating residual metabolites Such a restriction is naturally a desideratum for under these conditions the urinary output of the night period supplies a unit serviceable in arriving at a close and

6 The actual amount of urinary solids for the twenty-four hours represented by 25 would be 25X23 or 25X26, i e, between 58 and 65 gm This closely corresponds to the findings in actual determinations of the average twenty-four hour urinary solids Thus, Matthews places the figure at 61 gm Vierordt quotes the figure as 60 gm

Matthews, Albert P *Physiological Chemistry*, 1915, p 687

Vierordt, H *Anatomische, physiologische and physikalische Daten und Tabellen*, New York, Wm Wood & Co, 1906, p 330

fair index of the rate of excretion of the metabolites formed from the food ingested during the day

For proper emphasis of the cogent concept underlying this day to night ratio one might state the idea thus the day to night ratio of eliminated solids represents the quantum of excretory substances eliminated during the day, the said quantum expressed in units of nocturnal residual metabolites (solids of night period)

Thirdly A modification of Factor I, the incorporation of the element of actual total twenty-four hour solids eliminated If we sought to establish indices of renal function in terms of the rate of elimination of metabolites such a rate would have to be expressed with reference to some unit of quantity

With all other things equal, the more solids the kidney is called upon to excrete the more time it needs for such elimination and the kidney cannot be expected to maintain the same ratio of day to night elimination as the total twenty-four hour output fluctuates

This statement necessarily demands more than the mere expression of it for its acceptance We might hasten to add that we are not aware of any physiologic principle of renal activity that compels this conclusion, nor naturally of any principle that forbids it In the absence of guiding generalizations we must fall back upon our data for substantiation We find on examination of our data for the normal cases the existence of a negative correlation between the magnitudes expressive of Factor I and of the total twenty-four hour solids This is to say, that the mutual relationship between these two characteristics of the reaction is such, that, in general tendency, the higher, numerically, Factor I, the lower the total twenty-four hour solids Our data, therefore, permit of the implication made in the preceding paragraph

We have, therefore, taken 25,⁶ which appears to be about the average total solid output on a full diet in the average normal individual, as the unit of quantity We have in each case multiplied Factor I by the ratio $\frac{\text{total 24 hr solids}}{25}$ The resulting product we have designated Factor II To the extent to which this latter ratio (total twenty-four hour solids divided by 25) approximates unity, Factor II will numerically approximate Factor I, falling below it as the ratio falls below one, exceeding it as the ratio increases above one Factor II, then, involves the added element of the absolute quantity of solids excreted

Fourthly A further introduction of the element of concentration of urinary solids

The concentration of the eliminated solids is a feature of kidney activity long shown to be significant in the study of renal

function Factor II, has, therefore, been multiplied by the ratio $\frac{1500}{\text{total volume 24 hours}}$ (1500 c c being selected as an acceptable (upper range) average daily output) This product has been called Factor III ⁷

So then, as the total volume exceeds 1,500 c c, Factor III becomes numerically smaller than Factor II, and as it falls below 1,500 c c it increases in like ratio above Factor II

It may be emphasized at this point that with the introduction of these several "factors" we are not seeking to express or establish any mathematical law of kidney excretion nor are we desirous of adorning the physiology of kidney function with the cloak of mystic numbers. Primarily, our purpose is to study the rate of excretion of urinary solids. This rate we have chosen to estimate in terms of the ratio of the solids excreted during the day to that excreted at night. Inasmuch as such a ratio would be affected by the total quantity of solids excreted as well as by the total volume of fluid and inasmuch as it was necessary, if we desired to secure comparable ratios that constants be introduced about which these ratios were to swing, Factors II and III were introduced. We arbitrarily and empirically selected 25 ⁶ as the total solid constant and 1,500 as the total volume constant both of which figures fairly well represent the average output respectively of solids and fluids. Since with the increase of the total solid output the day to night

⁷ That Factor III is equivalent to Factor I, multiplied by the sp gr of the total twenty-four hour specimen, times a constant, can be seen from the following

$$\text{Factor I} = \frac{\text{Sp gr} \times \text{Vol (of day specimen)}}{\text{Sp gr} \times \text{Vol (of night specimen)}}$$

$$\text{Factor II} = \frac{\text{Factor I} \times \text{Sp gr} \times \text{Vol (of 24 hour specimen)}}{25}$$

$$\begin{aligned} \text{Factor III} &= \text{Factor II} \times \frac{1500}{\text{Vol. (of 24 hr Spec)}} \\ &= \text{Factor I} \times \text{Sp. Gr} \times \frac{\text{Vol. (of 24 hr Spec)}}{25} \times \frac{1500}{\text{Vol. (of 24 hr. Spec.)}} \end{aligned}$$

$$\begin{aligned} & \quad \quad \quad \text{[constant]} \\ & \quad \quad \quad \parallel \\ &= \text{Factor I} \times \text{Sp gr (24 hr spec)} \times \frac{1500}{25} \end{aligned}$$

The constant $\frac{1500}{25}$ is a ration expressive of a function of a concentration since the 1500 represents volume and the twenty-five solids contained therein. Factor III, therefore, falls back for its derivation on Factor I, and results from introducing into this latter "factor" the element of concentration of solids in the urine. Thus, both Factors II and III are derived with equal directness from Factor I

ratio tends on the whole to become numerically smaller we introduced the solid constant in a manner to counterbalance this reduction, by Factor II increasing above Factor I, in direct proportion to the increase of total twenty-four hour solids over 25⁶. Furthermore, since a diminution in the amount of fluid supplied to the kidney has a tendency to hold back urinary solids as well, reducing the total solid output and likewise delaying the rate of excretion of solids, the volume constant⁷ was introduced in a fashion to offset this interference by Factor III increasing numerically above Factor II in proportion to the reduction of the twenty-four hour volume below 1,500 cc. In a word, our desire has been to secure numerical ratios representing the rate of

TABLE 2—NON-NEPHRITIC CASES

Case	Maximum Sp Gr	Maximum Sp Gr Variation	Night Urine			Total N Gm	Total 24 Hr Solids*	Factor		
			Volume	Sp Gr	N Conc %			I	II	III
1	1 020	10	210	1 018	0 74	9 43	27 9	6 40	9 60	7 60
2	1 020	6	225	1 022	0 59	11 3	28 47	4 50	5 10	4 60
3	1 023	10	225	1 017	1 25	9 7	24 67	5 45	5 40	5 70
4	1 016	10	133	1 020	0 90	7 83	20 45	6 70	5 50	5 05
5	1 031	16	268	1 022	1 69	15 67	29 17	3 96	4 60	5 60
6	1 022	10	212	1 030	1 70	13 2	31 2	3 91	4 87	4 47
7	1 025	14	255	1 027	1 21	9 3	30 5	3 36	4 76	3 78
8	1 032	18	320	1 028	1 20	10 00	33 58	2 75	3 80	4 10
9	1 017	6	218	1 023	0 70	8 47	22 7	3 55	3 23	3 15
10	1 024	15	170	1 030	1 43	7 62	20 27	2 98	2 42	2 50
11	1 030	23	165	1 035	1 81	9 30	23 1	3 02	2 80	3 41
12	1 025	16	185	1 028	1 28	8 50	21 1	3 06	2 58	3 00
13	1 026	15	281	1 024	1 38	11 5	22 45	2 32	2 08	2 65
14	1 022	12	300	1 028	1 48	10 0	26 11	2 10	2 20	2 31
15	1 017	8	380	1 017	0 68	7 89	23 38	2 45	2 20	2 12
16	1 026	16	220	1 026	1 24	8 39	20 50	2 60	2 08	2 67
17	1 025	15	236	1 025	1 58	12 13	24 52	3 15	3 10	3 18
18	1 022	13	224	1 026	1 27	9 36	25 48	3 87	3 42	2 92
19	1 030	10	164	1 031	1 97	13 24	21 77	3 22	2 80	4 53
20	1 021	8	209	1 028	1 53	9 27	23 91	3 08	3 07	3 76
21	1 020	10	265	1 016	0 72	8 59	19 77	3 65	2 90	2 48
22	1 034	12	270	1 030	1 49	10 88	24 83	2 06	2 05	3 31
23	1 018	8	235	1 027	1 26	10 21	31 35	3 94	4 92	3 64
24	1 030	18	564	1 018	1 02	13 46	33 90	2 38	3 20	2 46
25	1 022	10	340	1 026	1 09	10 79	33 06	2 77	3 61	2 91

* The numerical values for the total solids here given represent the estimated solids divided by the constant multipliers 23 or 26, the constants involved in estimating urinary solids from a knowledge of the volume and specific gravity of a specimen

excretion of urinary solids, the said ratios being corrected for the total quantity of solids and fluids⁷ eliminated in the twenty-four hours and such corrections have been introduced in the form of Factors II and III. Just what such an attempt secured we submit below.

In addition to the estimation of the three "factors" as explained above, we have included in the tabulations given the significant data of each test diet reaction sufficient to permit of their interpretation in the terms adopted by Mosenthal. The maximum specific gravity of the two hour specimens, the amount of night volume, its specific gravity and its nitrogen concentration supply the data upon which his interpretation of the reaction is based. In addition, the tables contain the estimated and total nitrogen determinations.

In Tables 2 and 3 we have tabulated the test meal reaction of fifty unselected non-nephritic cases. In Table 2 are assembled those all of whose "factors" numerically equal or exceed two. In Table 3 are listed those in which at least one factor equaled or exceeded two. In the reactions of Table 2, we find that the variations in specific gravity of the two hour specimens are very good, the night urine tends to remain below 400 c c with a specific gravity usually well above 1.018 and a nitrogen concentration about 1 per cent, all these features of the reaction early looked on by Mosenthal as indicative of the certainly normal response. In Table 3, the reactions also quite well approximate the specifications for the normal reactions as laid down by him. Here, however, several

TABLE 3—NON-NEPHRITIC CASES

Case	Maxi- mum Sp Gr	Maxi- mum Sp Gr Vari- ation	Night Urine			Total N Gm	Total 24 Hr Solids*	Factor		
			Volume	Sp Gr	N Conc %			I	II	III
1	1.022	6	405	1.020	1.28	13.3	23.69	1.92	1.82	2.22
2	1.023	6	315	1.025	1.46	9.7	22.38	1.85	1.66	2.33
3	1.025	17	470	1.018	0.87	10.88	28.05	2.32	2.60	1.95
4	1.024	14	600	1.015	0.74	10.6	26.94	1.99	2.14	1.71
5	1.029	4	400	1.027	1.27	10.2	20.75	1.48	1.58	2.43
6	1.021	13	307	1.024	1.29	8.8	22.8	2.08	1.90	1.82
7	1.020	8	412	1.018	0.83	9.0	23.2	2.12	1.97	1.97
8	1.031	3	575	1.018	0.99	12.40	25.18	1.44	1.48	2.02
9	1.014	8	520	1.020	0.85	11.28	31.6	2.03	2.56	1.54
10	1.030	10	300	1.020	1.26	8.30	16.48	1.75	1.45	2.43
11	1.030	15	400	1.016	0.85	8.58	20.86	2.26	1.83	2.10
12	1.020	6	325	1.020	1.07	8.26	20.48	2.16	1.77	2.30
13	1.031	1	170	1.031	2.42	10.93	14.54	1.74	1.01	3.20
14	1.020	11	200	1.021	0.68	9.87	13.76	2.28	1.25	1.68
15	1.022	10	450	1.014	0.67	6.46	20.19	2.20	1.76	2.28
16	1.029	4	325	1.029	1.19	8.39	23.43	1.47	1.38	2.45
17	1.022	4	265	1.025	1.48	8.55	18.03	1.73	1.25	2.23
18	1.016	5	180	1.024	1.17	6.20	17.93	3.16	2.28	1.86
19	1.014	7	370	1.016	0.76	6.95	18.80	2.17	1.60	1.58
20	1.026	12	330	1.026	1.19	9.20	23.60	1.75	1.65	2.35
21	1.025	10	790	1.017	0.70	14.9	34.68	1.59	2.21	1.74
22	1.033	5	342	1.031	1.50	11.30	24.06	1.27	1.22	2.30
23	1.023	14	490	1.020	0.91	11.2	28.74	1.94	2.23	1.82
24	1.020	11	244	1.020	1.05	6.91	17.62	2.60	1.83	2.20
25	1.024	20	185	1.032	1.42	7.23	17.22	1.92	1.32	2.02

striking deviations from the average normal reactions appear, increase of night volume above 400 c c (Cases 4, 8, 9, 21), reduction of specific gravity of night specimen below 1.018 (Cases 4 and 15). That extra-renal factors can and do operate in normal individuals to produce these deviations from the norm we have demonstrated in another report¹ and appreciation of this has led Mosenthal³ to revise his original standards of normality.

From a perusal of Table 2, then, it appears that in normal individuals with definitely normal test meal reactions the ratio of solids passed during the day twelve hours to that passed during the night twelve hours is at least 2:1, and further, that the total solids are sufficient in amount and the total volume of urine for the day sufficiently low (i.e.,

concentration of solids sufficiently high) to keep Factors II and III as well, numerically above two. *Or, to express it in terms of clinical equivalents, the definitely normal reaction to the renal function test diet is an excretion of at least twice the amount of solids during the day as night with a total solid elimination of about 25^g and a total twenty-four hour volume of urine of not over 1,500 cc*

In Table 3 the responses to the test meal by normal individuals have been, on the whole, slightly poorer, and though all the "factors" have not reached 2, at least one of them has, and those "factors" that have failed to reach 2 are considerably above 1. It would appear, therefore, that in normal individuals even where extrarenal elements might have entered to prevent a strictly normal test meal reaction, the introduction

TABLE 4—CARDIO-VASCULAR CASES

	Maximum Sp Gr	Maximum Sp Gr Variation	Night Urine			Total N Gm	Total 24 Hr Solids	Factor			Diagnosis
			Vol ume	Sp Gr	N Conc %			I	II	III	
1	1.015	6	870	1.011	0.56	10.3	18.57	0.94	0.70	0.60	Chronic endocarditis, arteriosclerosis, myocarditis
2	1.017	2	202	1.020	1.14	5.04	7.8	0.78	0.25	0.00	Chronic cardiac insufficiency, myocarditis
3	1.029	6	570	1.025	1.01	11.9	25.35	0.95	0.96	1.36	Hypertensive cardiovascular disease
4	1.021	10	332	1.021	0.98	6.28	14.43	1.01	0.61	1.25	Hypertensive cardiovascular disease
5	1.025	7	198	1.024			10.7	1.27	0.55	1.70	Chronic cardiac insufficiency, syphilis
6	1.024	6	601	1.018	0.52	7.72	25.20	1.41	1.43	1.48	Chronic cardiac insufficiency
7	1.023	2	1210	1.015	0.61	15.2	25.65	0.96	0.99	0.74	Cardiac insufficiency, eliminating edema
8	1.022	10	890	1.010	0.32	6.44	17.76	0.99	0.70	0.70	Chronic cardiac insufficiency, chronic endocarditis
9	1.028	8	300	1.025	1.31	7.37	12.57	0.68	0.34	0.97	Chronic cardiac decompensation
10	1.022	7	300	1.020	0.83	5.32	12.07	1.01	0.49	1.18	Chronic myocarditis
11	1.020	10	632	1.018	0.61	7.43	22.06	0.93	0.83	0.92	Cardiac insufficiency, mitral stenosis, auricular fibrillation
12	1.026	5	397	1.018	0.77	8.88	17.57	1.46	1.03	1.56	Aortic aneurism
13	1.021	9	560	1.016	0.58	9.38	21.35	1.38	1.18	1.37	Aortic aneurism

of the several salient features of renal response in the manner attempted has succeeded in bringing at least one "factor" numerically above 2. Thus this procedure for estimating the result of the test meal reaction seems able to correct for the interfering influences upon the reaction consequent upon responses of the kidney to stimuli from uncontrolled sources, for with oliguria there is compensatory increase in concentration of solids and with polyuria an approximately equivalent reduction in solid concentration.

In Tables 4, 5 and 6, are given the findings in cardio-vascular, cardio-nephritic and nephritic cases.

Cardiovascular Cases—In the patients with well marked cardio-vascular disease, as was the case with the patients here listed, the

nutrition of the kidney is markedly interfered with and the renal function similarly disturbed. Where through kidney congestion and circulatory embarrassment there is interference with the elimination of water, as in Cases 2, 4, 5, 9, and 10 (Table 4), the excretion of solids is much delayed and the total amount of solids eliminated in the twenty-

TABLE 5—CARDIO-NEPHRITIC CASES

	Maxi- mum Sp Gr	Maxi- mum Sp Gr Vari- ation	Night Urine			Total N Gm	Total 24 Hr Solids	Factor			Diagnosis
			Vol- ume	Sp Gr	N Conc %			I	II	III	
1	1 029	4	350	1 028	1 77	11 8	19 46	0 98	0 76	1 63	Chronic cardiac insuffi- ciency, chronic nephritis
2	1 081	10	116	1 081	1 68	5 48	11 35	2 16	0 98	3 78	Chronic hypertensive car- diovascular, chronic ne- phritis
3	1 025	7	634	1 021	0 78	10 1	27 7	1 09	1 21	1 34	Arteriosclerosis, cardiac in- sufficiency, chronic diffuse nephritis
4	1 017	3	800	1 015	0 61	9 68	20 45	0 70	0 57	0 65	Cardiovascular disease, dif- fuse nephritis
5	1 016	6	233	1 016	0 84	4 10	7 40	0 98	0 30	0 85	Chronic cardiac insuffi- ciency, diffuse nephritis
6	1 012	3	1014	1 009	0 39	8 97	19 9	1 19	0 95	0 68	Cardiac decompensation, chronic interstitial ne- phritis
7	1 021	2	212	1 023	1 14	5 86	13 07	1 67	0 87	2 14	Chronic cardiovascular dis- ease, chronic diffuse ne- phritis
8	1 010	4	1056	1 009	0 28	7 28	18 67	0 97	0 72	0 49	Chronic cardiac insuffi- ciency, chronic diffuse ne- phritis
9	1 014	4	535	1 012	0 55	5 72	11 95	0 86	0 41	0 62	Aortic insufficiency, chronic diffuse nephritis
10	1 013	2	725	1 010	1 25	14 67	19 50	1 56	1 21	1 10	Cardiovascular disease, chronic diffuse nephritis
11	1 018	8	875	1 008	0 32	5 08	13 87	0 98	0 54	0 58	Chronic endocarditis, arte- riosclerosis, chronic diffuse nephritis
12	1 011	1	870	1 010		6 15	15 71	0 81	0 54	0 49	Chronic endocarditis, arte- riosclerosis, chronic diffuse nephritis
13	1 020	13	615	1 017	0 55	7 18	20 17	0 93	0 75	0 72	Chronic cardiac insuffi- ciency, chronic glomerulo- nephritis
14	1 020	10	740	1 020	0 58	9 0	27 21	0 84	0 92	0 79	Chronic cardiac insuffi- ciency, chronic nephritis
15	1 025	6	460	1 020	0 60	5 47	18 14	0 97	0 71	1 22	Chronic cardiac insuffi- ciency, chronic glomerulo- nephritis
16	1 010	4	1056	1 009	0 28	7 28	18 67	0 97	0 72	0 49	Chronic cardiac insuffi- ciency, chronic diffuse ne- phritis
17	1 016	3	570	1 014	0 64	7 90	15 47	0 93	0 67	0 78	Aortic insufficiency, chronic diffuse nephritis
18	1 014	1	481	1 012	0 42	4 88	14 52	1 52	0 88	1 18	Chronic endocarditis, chronic diffuse nephritis
19	1 012	2	710	1 010	0 41	5 69	14 87	1 09	0 67	0 70	Chronic endocarditis, chronic diffuse nephritis
20	1 020	12	1450	1 010	0 28	7 93	28 38	0 96	1 09	0 55	Chronic endocarditis, chronic cardiac insuffi- ciency

four hours is well below normal. Nitrogen excretion is least hampered which fact in conjunction with the oliguria results in the excretion of nitrogen in good concentration and with a night specimen well below 400 c c. However, in cases in which apparently arteriosclerotic changes in the kidney predominate, there is frequently definite interference with the power for elimination of nitrogen in good concentration in which

cases the reactions to the test diet approximate those of interstitial nephritis. Under these conditions the night specimens are increased in volume and nitrogen concentration is low (Cases 1 and 8). Where beginning cardiac compensation is evidencing itself by the elimination of edema, the total solids and fluids excreted are much increased, but the rate of elimination of the solids as expressed in terms of the ratio of the day to night solids is well below that in normal cases (Case 7). Thus, be the test meal reaction one of oliguria from renal congestion, or that of the arteriosclerotic kidney simulating the reaction of interstitial nephritis or that of polyuria from the elimination of edema, the rate of solid excretion as estimated in the fashion we have herein attempted is very considerably reduced below that found in our normal cases. The numerical values of all three "factors" are well below 2, all tending, on the whole, to border about and usually below 1.

TABLE 6—NEPHRITIC CASES

	Maxi- mum Sp Gr	Maxi- mum Sp Gr Varia- tion	Night Urine			Total N Gm	Total 24 Hr Solids	Factor			Diagnosis
			Vol ume	Sp Gr	N Conc %			I	II	III	
1	1.021	8	615	1.017	0.79	13.6	24.77	1.38	1.36	1.39	Chronic interstitial nephritis
2	1.015	2	1185	1.012	0.41	10.74	33.38	1.35	1.80	1.05	Chronic interstitial nephritis
3	1.022	4	390	1.018		6.8	14.83	1.12	0.67	1.27	Chronic parenchymatous ne- phritis
4	1.018	8	1336	1.014	0.88	10.78	40.32	1.17	1.89	0.96	Chronic interstitial nephritis
5	1.017	4	515	1.016	0.61	6.0	18.38	0.88	0.64	0.73	Chronic interstitial nephritis
6	1.013	2	914	1.012	0.76	13.9	22.56	1.00	0.90	0.73	Chronic interstitial nephritis
7	1.020	8	460	1.025	0.44	4.86	19.38	0.69	0.53	0.73	Chronic interstitial nephritis
8	1.023	5	460	1.020	0.97	9.11	19.49	1.11	0.87	1.30	Chronic diffuse nephritis
9	1.016	4	700	1.011	0.33	4.7	15.87	1.06	0.68	0.80	Chronic interstitial nephritis
10	1.020	13	995	1.011	0.44	8.16	17.93	0.65	0.47	0.46	Chronic glomerulo nephritis
11	1.030	12	888	1.010	0.46	8.4	18.0	1.03	0.75	0.83	Chronic interstitial nephritis
12	1.019	2	625	1.020	1.10	14.0	23.88	0.91	0.87	1.05	Chronic diffuse nephritis
13	1.029	22	814	1.012	0.58	9.8	22.1	1.20	1.11	0.88	Chronic diffuse nephritis
14	1.015	12	810	1.011	0.47	9.1	18.43	1.07	0.79	0.60	Chronic diffuse nephritis
15	1.028	15	1020	1.012	0.43	7.44	19.93	0.63	0.50	0.50	Chronic glomerulo nephritis
16	1.017	3	700	1.016	0.62	8.92	22.79	1.03	0.94	0.89	Subacute glomerulo nephritis
17	1.009	1	832	1.009	0.40	5.6	11.89	0.59	0.28	0.32	Chronic interstitial nephritis
18	1.029	15	899	1.010	0.67	10.1	17.53	0.95	0.67	0.79	Chronic interstitial nephritis
19	1.024	7	760	1.014	0.90	11.46	18.56	0.74	0.48	0.64	Chronic interstitial nephritis
20	1.028	2	590	1.020	1.2	10.9	19.01	0.61	0.46	0.81	Chronic interstitial nephritis
21	1.025	6	670	1.017	0.58	8.8	22.93	1.01	0.93	1.16	Chronic interstitial nephritis

Cardio-nephritic and Nephritic Cases—Be the functional reaction of the kidney more indicative of the circulatory disturbance in the cardio-nephritic or of his renal lesion—be the renal condition one interfering primarily with the salt and water or with the nitrogen elimination, the rate of excretion of the urinary solids is much delayed and the total amount of solids, on the whole, definitely reduced below that found in normal individuals. Usually all three 'factors' as calculated fall numerically well below 2, a high percentage of them dropping below 1. The reactions, likewise, viewed in the terms of specific gravity variation of the two hour specimens and character of night urine, correspond with those found and described as indicative of renal insufficiency. With

renal involvements, then, there appears a definite delay in the excretion of urinary solids—a definite increase relatively and at times absolutely in the quantity of solids excreted at night

In Tables 7 and 8, we have grouped reactions to the test diet given to the same individual on two, three or more days, many of the tests in the same individual being given on consecutive days. It appears, therefrom that the results expressed in terms of the rate of solid excretion by means of the three 'factors' show very acceptable consistency for the same individual. In the group of normal cases, the same individual in several test meal reactions, be the reaction characterized by total oliguria with high concentration of all specimens (S 299) or by high night volume with reduced specific gravity

TABLE 7—REPEATED TEST MEALS IN NON-NEPHRITIC INDIVIDUALS

		Maxi- mum Sp Gr	Maxi- mum Sp Gr Varia- tion	Night Urine			Total N Gm	Total 24 Hr Solids	Factor		
				Vol ume	Sp Gr	N Conc %			I	II	III
S 207	12/ 6/16	1 022	10	340	1 026	1 09	10 79	33 06	2 77	3 61	2 01
S 294	2/ 5/17	1 023	7	540	1 018			30 79	2 18	2 68	2 54
S 296	2/ 6/17	1 025	9	380	1 024	0 72	11 3	30 29	2 31	2 80	2 98
S 297	2/ 7/17	1 026	11	610	1 016	1 1	18 8	32 5	2 33	3 02	2 68
S 298	2/ 8/17	1 027	4	325	1 025	0 77	7 30	25 2	2 09	2 08	2 94
S 299	2/ 9/17	1 029	4	400	1 027	1 27	10 2	26 7	1 48	1 58	2 43
B 313	2/13/17	1 031	11	303	1 029	1 20	10 5	21 41	2 31	1 98	2 54
B 314	2/14/17	1 030	4	276	1 031	1 40	10 0	25 2	1 95	1 06	3 44
B 315	2/15/17	1 033	5	342	1 031	1 50	11 3	24 6	1 27	1 22	2 30
O 318	2/13/17	1 020	5	630	1 019	0 80	14 5	44 82	2 74	4 90	2 82
O 319	2/14/17	1 027	4	280	1 029	1 70	16 8	29 35	2 61	3 06	4 50
O 320	2/15/17	1 025	10	790	1 016	0 70	14 9	34 68	1 59	2 21	1 74
O 321	2/16/17	1 029	9	290	1 029	1 92	14 80	25 06	1 98	1 99	3 10
D 346	3/ 5/17	1 025	18	315	1 023	1 10	7 8	21 55	1 97	1 69	1 86
D 347	3/ 6/17	1 021	14	210	1 027	1 20	7 7	19 67	2 47	1 94	2 01
D 374	3/20/17	1 019	13	375	1 019	0 93	8 8	19 40	1 72	1 33	1 43
D 375	3/21/17	1 020	16	320	1 022	1 15	9 4	18 88	1 68	1 26	1 33
D 376	3/22/17	1 023	19	285	1 025	1 3	9 2	18 48	1 59	1 17	1 33
N 258*	1/24/17	1 029	3	500	1 025			29 57	1 36	1 58	2 10
N 263*	1/26/17	1 023	10	340	1 018			17 62	1 92	1 35	2 60
E 276*	1/31/17	1 023	15	550	1 010			20 27	2 68	2 18	2 32
E 285*	1/28/17	1 033	10	212	1 024			23 16	2 48	2 28	3 88

* No regular test meal, subjects on their ordinary diets

(S 297, O 320), or by the nitrogen concentration in the night specimens considerably above 1 per cent or below it (O 320 or O 321)—supplied "factors" one of which was numerically at least equal to 2. So here too, it likewise appears that for the same non-nephritic individual on repeated examinations, even though successive reactions significantly vary in character of night specimen or two hourly specimens (S 296 and 297—I 320 and O 321) the "ratio-factor" and its "corrective factors" still produce a numerical product of at least 2 in one of the three factors. It is to be noted, too, that such was the case in individuals who were fed no special diets. Subjects N and E partook of their customary

diets In subject E's reactions is to be observed the marked variation in the character of the night specimens of his two test meals, in one an increased night volume with a very low specific gravity and in the other a scanty night volume with a high specific gravity and this with no appreciable difference in both test meal reactions when considered in terms of the rate of excretion of urinary solids Subject D succeeds but once in five test meals in securing "factors" numerically above 2 She reacts rather constantly below the normal so far as the rate of urinary solids excretion is concerned, although her reactions, as viewed from the character of the volume and specific gravity of the day

TABLE 8—REPEATED TEST MEALS IN NEPHRITIC INDIVIDUALS

		Maxi- mum Sp Gr	Maxi- mum Sp Gr Vari- ation	Night Urine			Total N Gm	Total 24 Hr Solids	Factor		
				Vol ume	Sp Gr	N, Conc %			I	II	III
O 352	3/ 5/17	1 022	4	540	1 013	0 54	6 0	16 1	1 29	0 83	1 24
O 362	3/12/17	1 022	11	1101	1 009	0 26	5 8	21 8	1 14	0 97	0 80
O 363	3/13/17	1 022	5	870	1 011	0 36	5 4	17 03	0 78	0 58	0 20
O 366		1 024	6	1085	1 007			16 01	1 10	0 71	0 71
L 343	2/23/17	1 029	15	899	1 010	0 67	10 1	17 53	0 95	0 67	0 79
L 344	3/ 1/17	1 024	7	760	1 014	0 90	11 4	18 56	0 74	0 48	0 64
L 345	3/ 2/17	1 028	2	590	1 020	1 2	10 9	19 01	0 61	0 48	0 85
T 65	5/20/16	1 020	13	615	1 017	0 55	7 18	20 17	0 93	0 75	0 72
T 67	5/27/16	1 024	10	430	1 014	0 40	4 25	17 44	1 91	1 31	1 89
T 81	6/15/16	1 025	6	460	1 020	0 60	5 47	18 14	0 97	0 71	1 22
T 127	9/ 1/17	1 019	9	750	1 011	0 38	7 16	16 14	0 96	0 62	0 73
T 134	9/26/17	1 017	7	725	1 014	0 50	8 48	23 8	1 37	1 31	1 11
T 158	10/30/16	1 020	10	725	1 016	0 50	7 32	22 48	0 94	0 84	0 82
F 177	11/11/16	1 028	15	1020	1 012	0 43	7 44	19 93	0 63	0 50	0 50
F 186	11/18/16	1 020	13	995	1 011	0 44	8 16	17 93	0 65	0 47	0 46
F 199	11/28/16	1 018	8	1250	1 012	0 62	11 07	26 09	0 74	0 77	0 57
H 30	4/10/16	1 013	2	940	1 012	0 76	13 9	22 56	1 0	0 90	0 76
H 40	4/21/16	1 015	2	1185	1 012	0 41	10 74	33 38	1 35	1 80	1 05
H 49	5/ 3/16	1 010	1	970	1 010	0 39	9 02	24 7	1 55	1 53	0 92

and night specimens, are consistently normal We deem it somewhat premature to attempt to further discuss at this time any disagreement in findings in the same individual by the two methods of test meal interpretation Sufficient for our present purpose is the observation that the rate of solid excretion method tends, at least, to be consistent with itself Likewise, for the nephritic cases a similarly persistent constancy in reaction by the same individual manifests itself As in the table of the nephritic groups above outlined the "factors" tend to amount numerically to about, and, in greatest frequency, to below 1

SUMMARY AND CONCLUSIONS

In a study of over 300 renal function test meals we have been impressed with the operation of extrarenal influences on the reaction which in many cases interfered with the reliability of interpretations of the findings expressed in terms of the specific gravity variation of

the two hour specimens and character of the night urine. We endeavored to circumvent this interference which, we suspected because of an earlier study was due to extraneous factors influencing the fluid supply to the kidney, by attempting to translate the renal function test meal findings into terms of the urinary solids eliminated. The solids were estimated from the specific gravity and volume of the specimens and the following features of the excreted solids studied

1 The total twenty-four hour estimated urinary solids. This amounted on the average in normal cases to about 25 (this figure to be multiplied by constant 2.3 or 2.6 to secure in gm. the absolute amount of solids present). In renal, cardiorenal and cardiac decompensation cases the urinary solids eliminated during the twenty-four hours usually fell well below this figure.

2 The rate of excretion of urinary solids expressed in terms of the ratio of the solids eliminated during the day to that of the night. To this ratio was applied the designation Factor I.

3 The introduction of corrective checks upon Factor I for the absolute twenty-four hour solids and volume eliminated and in a manner to allow for a delay in rate of solid excretion consequent upon an increase in total solid output or reduction in amount of fluid excreted, thus permitting of comparisons between the numerical findings expressive of the rate of excretion in all our reactions. These checks are introduced as Factors II and III.

$$\text{Factor II equaling Factor I} \times \frac{\text{total 24 hr solids}}{25}$$

$$\text{Factor III equaling Factor II} \times \frac{1500}{\text{total 24 hr volume}}$$

Application of this method of interpretation showed that distinctly normal persons, with the test meal used, excrete at least twice as much urinary solids during the day as during the night with a total twenty-four hour solid elimination in the neighborhood of (25)⁶ and a total volume of about 1,500 c c, so that all Factors I, II and III produce the numerical equivalent of at least 2.

In most cases in which influences might have entered to interfere with a normal response to the test meal, the corrective factors apparently operated to produce at least one "factor" numerically equal to 2.

For kidneys with defective function, either by virtue of renal, cardiac or cardiovascular disease, there is evidenced both a reduction in the total solids excreted in twenty-four hours and a marked delay in the rate of excretion as estimated in terms of the ratio of day to night elimination of urinary solids. Hence, in these cases all three

"factors" of the test meal reaction tend to be numerically well below two, ranging, as a rule, about 1 as a mean

This method of estimating the results of the renal function test meal presents as equally sharp a differentiation between the normal and subnormal functioning kidney as does the method based on a study of the specific gravity variation of the two hour specimen and character of night urine

It further appears to disclose greater constancy in reaction by the same individual, particularly in persons with normal or only slightly diseased kidneys, than does the former method

This method obviates the need for collecting urinary specimens at exactly two hour intervals

Where urinary specimens have been collected in two hourly periods during the day the results of the test can be judged by both methods of interpretation as we have been able to do with all our cases

The essential procedures in securing acceptable data for estimating the rate of solid excretion are (a) patient to abstain from food or drink between meals, (b) heaviest meal to be taken at noon, and (c) three hours be permitted to elapse after the evening meal before beginning collection of the night specimen

It was on the basis of this procedure with our test meals that the data above submitted were obtained

TERMINAL CARDIAC ARRHYTHMIAS

REPORT OF THREE CASES [†]

FRANCIS R DIEUAIDE, M D

AND

EDWARD C DAVIDSON, M D

BALTIMORE

Because of the frequency with which death of the individual is dependent on cardiac failure, the mode of death of the heart is a matter of considerable interest. Moreover, it is highly probable¹ that one of the mechanisms found to occur in gradual cardiac death, that is, ventricular fibrillation, is responsible for many sudden deaths of otherwise unknown cause, as suggested by McWilliam². At the present time, the electrocardiogram offers our best means of attacking this problem. Despite the interest in the subject and its importance, there are but few clinical records of terminal mechanisms and fewer of ventricular fibrillation. The paucity of clinical electrocardiographic records of lethal exitus needs no explanation. That ventricular fibrillation is not better known clinically results from the fact that this mechanism is necessarily fatal, if it persists beyond a very few minutes. Experimentally, the condition is rather well known³ since it can be produced without difficulty by faradization of the ventricles,⁴ by occlusion of a coronary artery and by intoxication with digitalis, potassium, chloroform and other agents. In experimental work, death often occurs with the onset of ventricular fibrillation preceded by no known cause. The onset of experimental fibrillation is usually gradual, following ventricular extrasystoles, ventricular tachycardia and a disturbance resembling flutter, in the order named. When fibrillation is established, there are scarcely any movements of the ventricles, the heart is distended and the blood pressure at zero. The galvanometric record is characteristic, showing continuous slow movements of the string which are grossly irregular in amplitude and rhythm, with minute irregular deviations which are perhaps traces of auricular

[†] From the Cardiographic Laboratory of the Johns Hopkins Hospital and University.

1 Lewis, Thomas. Mechanism and Graphic Registration of the Heart Beat, London and New York, 1920, p. 320.

2 McWilliam, J. A. On the rhythm of the Mammalian Heart, *J. Physiol.* **9** 167, 1888.

3 Levy, A. G. Genesis of Ventricular Extra-Systoles Under Chloroform etc., *Heart* **5** 299, 1913.

4 McWilliam, J. A. Fibrillar Contraction of the Heart, *J. Physiol.* **8** 296, 1887.

activity Recovery of the ventricles in experimental animals is not infrequently seen, and it is interesting that this occurs more often in smaller animals⁵ It is probable that the occurrence of recovery is related to the size of the heart and the mass of the tissue involved⁶

The earliest clinical record suggesting ventricular fibrillation is Hoffmann's⁷ This is taken by Lewis as a transitional stage of tachycardia antecedent to actual fibrillation The patient recovered Rohmer⁸ reported terminal records of three fatal cases of diphtheria in which complete dissociation occurred In 1912, Robinson⁹ presented a group of seven cases with fatal outcome, with an electrocardiographic study of exitus In two instances the records showed the occurrence of ventricular fibrillation after all signs of life recognizable clinically had ceased, and in one of these cases fibrillation was followed by a regular rhythm Various other arrhythmias, including heart block, ventricular extrasystoles and tachycardia, occurred, without any characteristic sequence It was noted that there was a tendency for the amplitude of R to diminish while its duration and the amplitude of T increased and R and T gradually became fused It is to be noted that these patients died as a result of acute infectious diseases without primary cardiac involvement There was no one point in the heart found to succumb last Halsey¹⁰ reported a terminal record of a patient ill with pneumonia, in which death coincided with the development of ventricular fibrillation Robinson and Bredeck¹¹ obtained a record showing ventricular fibrillation, or a stage just short of that condition, from a patient suffering from heart disease, who recovered and lived thirty hours after the cessation of fibrillation It is of interest that the records of this case showed ventricular extrasystoles with marked variation in the type of the Q R S complex probably indicating a high degree of irritability and abnormal paths of conduction

The present cases are reported because of the occurrence of interesting arrhythmias determined electrocardiographically shortly before and during the interval immediately preceding death

REPORT OF CASES

CASE 1—W W, male, colored, aged 48, first admitted Oct 16, 1920 and discharged Oct 27, 1920

5 Gunn, J A Ventricular Fibrillation in the Rat's Heart, *Heart*, **5** 1, 1913
6 Garrey, W E Nature of Fibrillary Contraction of the Heart, *Am J Physiol* **33** 397, 1914

7 Hoffmann A Fibrillation of the Ventricles, *Heart*, **3** 213, 1911

8 Rohmer, P Ueber das Elektrokardiogramm des Diphtherieherztodes *Munchen med Wchnschr* **58** 2358, 1911

9 Robinson, G C Study with the Electrocardiograph of the Mode of Death of the Human Heart, *J Exper Med* **16** 291, 1912

10 Halsey, R H A Case of Ventricular Fibrillation, *Heart*, **6** 67 1915

11 Robinson, G C, and Bredeck, J F Ventricular Fibrillation in Man with Cardiac Recovery *Arch Int Med* **20** 725 (Nov) 1917

Complaint—Shortness of breath, cough and swelling of the ankles

Past History—Typhoid fever at 7 years of age Influenza in March, 1918 No history of rheumatic fever or sore throat Neisserian infection at 21 and again at 42 Nocturia for twenty years The patient denied syphilis and alcoholism

Present Illness—The onset was in January, 1918, with swelling of the ankles About two weeks after this the patient, on running for a car, became extremely dyspneic The dyspnea increased until he could not sleep lying down The patient suffered from cough, marked palpitation and periodic night sweats For two years puffiness of the eyelids had been quite noticeable in the morning The swelling of the ankles had been persistent

Physical Examination—Temperature, 98.8 F, pulse, 88, respiration, 22 Tonsils scarred No general glandular enlargement In the chest the signs of an old tuberculous process of the right upper lobe were present Many moist râles over both bases There was a forceful cardiac apical impulse in the fourth interspace, 14 cm to the left of the midsternal line There was a diastolic shock over the base No thrills felt The relative cardiac dullness extended 15 cm to the left and 4 cm to the right of the midsternal line There was retromanubrial dullness which reached 5 cm to each side of the midline The first sound was followed by a faint high pitched musical murmur at the apex The second sound was reduplicated The pulse was regular except for occasional extrasystoles The peripheral arteries were thickened and tortuous The blood pressure was 220/140 The liver edge was 8 cm below the costal margin No edema of the extremities was present Ophthalmoscopic examination showed the optic disks to have hazy margins The arteries were tortuous and the veins distended No hemorrhages or exudate

Laboratory Findings—Blood examination negative Blood urea, 18.5 mg Phenolsulphonephthalein, 60 per cent in two hours Wassermann reaction negative Urinary output, from 1,600 to 2,000 cc Sp gr, 1.010 to 1.028 Occasional trace of albumin, with a few hyaline and granular casts

Course—Uneventful No digitalis therapy Two electrocardiograms taken during this admission The patient was discharged free from cardiac failure

The patient was readmitted Dec 31, 1920 While out of the hospital he worked part of each day About three weeks after discharge his ankles began to swell, his dyspnea returned and his eyelids became puffy There were numerous attacks of vertigo

Physical Examination—Marked orthopnea Slight generalized edema The breath sounds were suppressed and many moist râles were heard over the base of both lungs The relative cardiac dullness measured 18 by 4 cm The pulse was regular Blood pressure 220/150 In the abdomen shifting dullness and a fluid wave were present Ophthalmoscopic examination showed several hemorrhages and some exudate

Laboratory Findings—Wassermann reaction negative Blood urea, from 35.8 to 47.2 mg Phenolsulphonephthalein, Jan 5, 1921, 47 per cent in two hours, Jan 18, 1921, 26 per cent in two hours Urinary output averaged 500 cc Albumin and casts constantly present

Course—The edema persisted The patient had constant tachycardia, nocturnal paroxysms of dyspnea and periods of Cheyne-Stokes respiration The temperature was often subnormal, and the blood pressure varied remarkably The patient grew progressively weaker, failing to respond to any therapy and died, March 21, 1921 During this admission electrocardiographic records were made twice, and finally just before and after the death of the patient

Necropsy Findings—The principal necropsy findings¹² may be summarized as follows The heart weighed 720 gm The myocardium showed yellowish

12 Necropsy findings in Cases 1 and 2 are presented by courtesy of Dr W G MacCallum, pathologist to the Johns Hopkins Hospital

white opacities scattered diffusely throughout, and distinct areas of depressed scars were seen in the surface. There were no valvular lesions observed. Many mural thrombi were present in the left ventricle. Both ventricles were dilated and hypertrophied. The aorta was not enlarged. It was fairly elastic but showed many raised plaques of beginning sclerosis. The lungs showed widespread bronchopneumonia. The spleen contained a recent organizing infarct. The kidneys had adherent capsules, there were several small infarcts and the cortex was thinned. The provisional diagnosis was chronic diffuse nephritis, cardiac hypertrophy and dilatation, Myocarditis with mural thrombi, Anasarca, Infarcts of spleen and kidneys, Edema of lungs, Bronchopneumonia, Pleurisy.

Electrocardiographic Records—Table 1 presents a summary of the electrocardiographic records of this patient. The first was taken Oct 16, 1920, the day of admission, and the second (Fig 1 A) twelve days later. These two records are similar in every respect and present no striking abnormality save a slight prolongation of the Q R S interval.

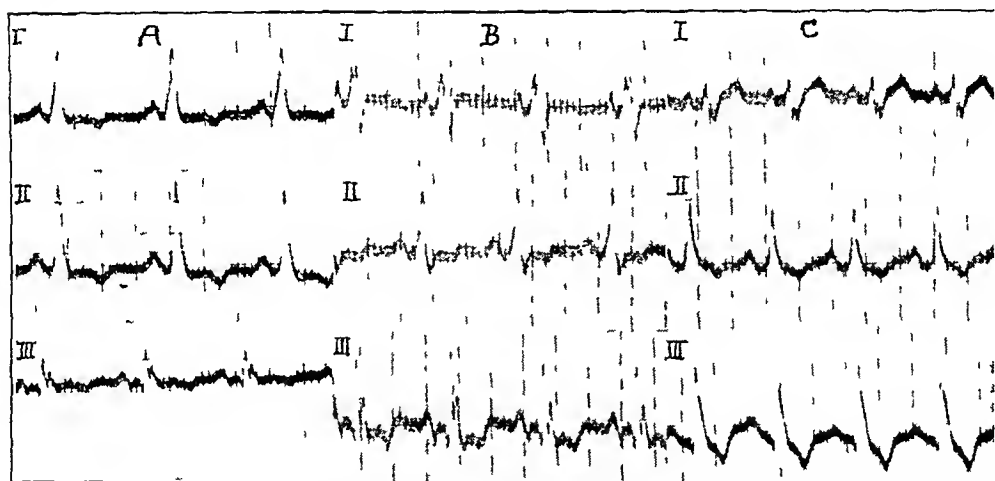


Fig 1—Case I A, Oct 28, 1920 B, Feb 1, 1921 C, March 21, 1921 (day of patient's death). The Q R S complex changes remarkably.

and notching of the R wave in Leads I and III. The third record, which was taken Jan 18, 1921, three months after the first, showed no significant change, except a longer Q R S interval, while in the fourth record (Fig 1 B), taken February 1, the notching in Leads I and III became more conspicuous. There is a remarkable progression in the type of the Q R S complex from the first to the fifth record. Analysis of the movements of the electrical axis shows that it pursues a fundamentally similar course throughout the records. The explanation and significance of the changes is at present obscure, but the hypothesis of increasing delay in the spread of the impulse throughout the left bundle would explain the outstanding features.

The remaining records were taken practically continuously on the day of the patient's death, beginning about ten or twelve minutes before all clinical signs of life ceased and ending shortly thereafter. Fig 1 C

shows the three usual leads at the beginning of this period. A marked sinus tachycardia, lengthened P-R and Q R S intervals are the chief new characteristics. The later figures are all of Lead III (the measurements in the table are of Lead III for all the records). The rate is gradually slowed from 120 to 65 in Figure 2. This figure is of great interest because of the changes in the "P" wave shown in it. In the early cycles of this record "P" is definitely positive, then it flattens out, disappears and finally is definitely negative, while the P-R interval is progressively shortened and the rate slowed. These changes may be interpreted either as a migration of the pacemaker within the sino-auricular node or as a shift from the sino-auricular to the auriculo-ventricular node¹². In Figure 3 but few P waves appear and those which are seen are at a very slow rate and irregular. It would seem that most of the complexes from this point on are nodal since they have approxi-

TABLE 1—MEASUREMENTS OF ELECTROCARDIOGRAMS OF CASE 1 (LEAD III)

Film No	Fig No	Date	Rate	Rhythm	Duration, Sec			Amplitude, Mm			Notes
					P R	QRS	Q T	P	R	T	
3077-1		10/16/20	85	Reg	0 15	0 08	0 32	0 75	6 0	-1 0	All "T's" —
3077-2	1A	10/28/20	94	Reg	0 15	0 09	0 33	1 0	5 0	-0 5	All "T's" —
3077-3		1/18/21	95	Reg	0 15	0 10	0 32	1 75	-9 0	-1 25	T ₁ +, T ₂ —, T ₃ —
3077-4	1B	2/ 1/21	105	Reg	0 15	0 10	0 29	1 75	12 0	-1 5	T ₁ +, T ₂ —, T ₃ —
3077-5	1C	3/21/21	120	Reg	0 16	0 10	0 27	1 0	11 5	-3 5	T ₁ +, T ₂ —, T ₃ —
3077-6	2	2/21/21	86-65	Irreg	0 21- 0 15	0 11	0 29	1 5	11 0	-3 0	Nodal rhythm
3077-7	3	3/21/21	60	Irreg	0 42	0 12	0 32	1 0- 0 0	13 0	-2 0	Nodal rhythm
3077-8	4	3/21/21	60	Irreg		0 12	0 44		3 0	4 0	Ventricular fibrillation
3077-9	5	3/21/21	55	Irreg		0 12	0 42		2 5	7 0- 3 0	Ventricular fibrillation
3077-10		3/21/21	47 5	Irreg		0 08	0 26		2 0- 0 0	0 5	

mately the normal outline. It is interesting that the coupling recorded is almost certainly due to occasional responses to impulses built up higher up in the auricular wall, since "P" waves appear at a constant P-R interval in connection with two of the coupled complexes, while the P which belongs with the third complex is hidden by the first R of that couple. Definite, moderately advanced ventricular fibrillation is shown in Figure 4 and again in Figure 5. Both periods of fibrillation are brief and are succeeded by series of abnormal complexes with no trace of P waves. The second period of fibrillation is a more advanced disorder than the first. With reference to the abnormal complexes, their origin cannot be definitely stated. The "T" waves are of high amplitude and positive whereas they were previously always negative. The tendency for the R and T waves to coalesce, pointed out by

¹² Lewis, T. Effect of Vagal Stimulation on Atrio-Ventricular Rhythm. *Heart* 5 247, 1914. Lewis, T., Meakins, J., and White, P. D. Excitatory Process in the Dog's Heart, *Phil Tr Roy Soc Lond*, B 205 375, 1914.

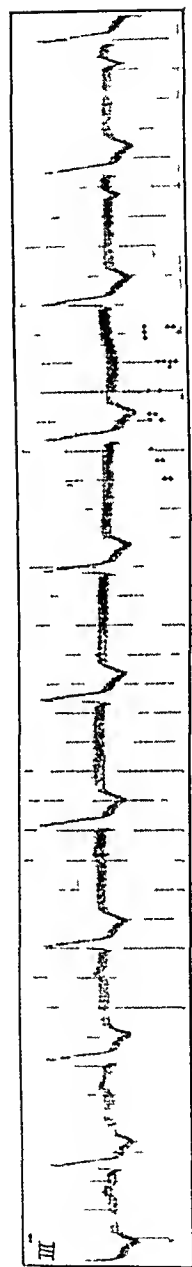


Fig 2—Case 1 Lead III Ten minutes before the patient's death Note the change in the P wave

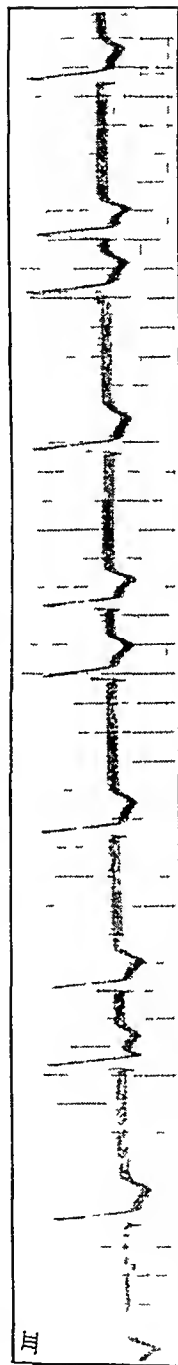


Fig 3—Case 1 Lead III A few seconds later than Figure 2 Nodal rhythm with occasional coupling due to impulses of auricular origin

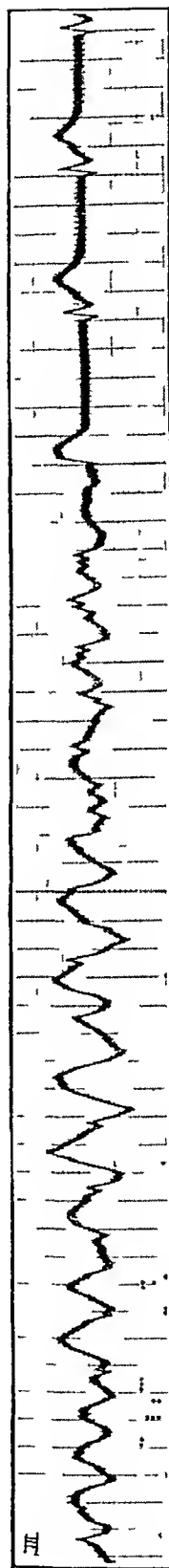


Fig 4—Case 1 Lead III Five minutes after Figure 3 Ventricular fibrillation followed by regular complexes of ventricular origin

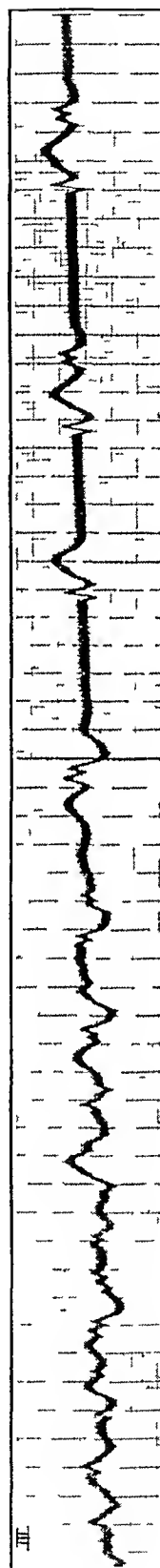


Fig 5—Case 1 Lead III A few seconds later Ventricular fibrillation followed by regular complexes similar to those of Figure 4 with occasional complexes of a second type

Robinson,⁹ is shown to some extent. The amplitude of the R waves gradually decreases more and more rapidly toward the end of the record. No attempt to record the exact instant of "clinical death" was made, but this occurred shortly before the last record was taken.

To summarize the electrocardiographic findings of this case, we may say that the rate gradually slowed, while the site of impulse

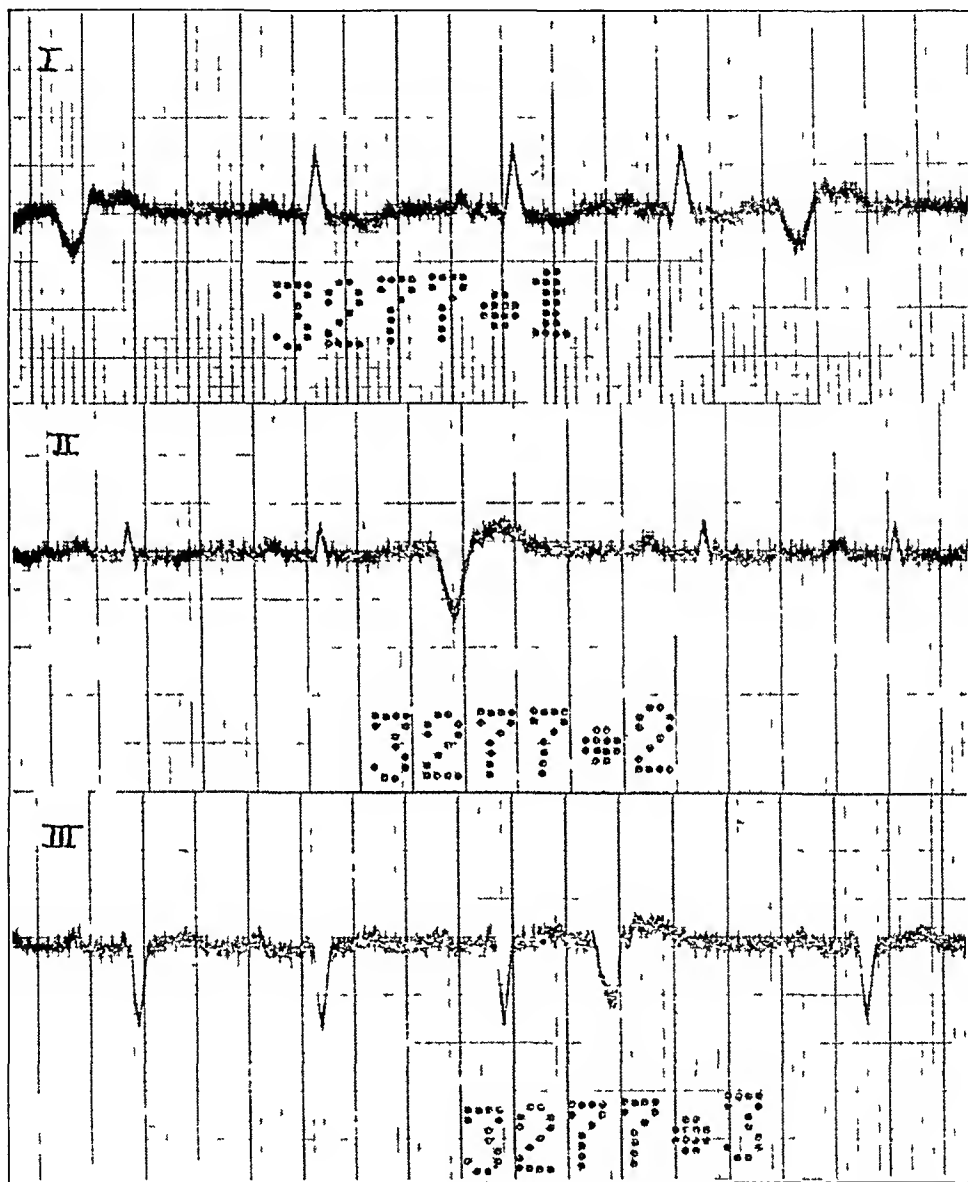


Fig 6—Case 2 April 25, 1921. Note the form of the normal and ectopic complexes.

formation either shifted within the sino-auricular node or passed to the auriculo-ventricular node, the conduction time decreased and then all definite evidence of auricular activity ceased. There were two brief periods of ventricular fibrillation interrupted by and followed by fairly regular but gradually slowing complexes due to impulses arising in an undefined part of the heart. The amplitude of the complexes

gradually decreased until it was very small when they finally ceased. The autopsy revealed no changes definitely associated with any of the electrocardiographic findings.

CASE 2—S B, male, colored, aged 48, admitted April 23, 1921

Complaint—Swelling of legs, shortness of breath and weakness

Past History—General health always good up to 1918. Neisserian infection in 1913. In 1918 the patient was admitted to this hospital with cough, shortness of breath and edema, and was found to have arteriosclerosis and hypertension. While in the hospital the patient suffered a right-sided hemiplegia, which cleared up. Between admissions the patient was able to work as usual.

Present Illness—Began three months before admission with cough, dyspnea and edema which grew worse until the patient became very weak.

TABLE 2—MEASUREMENTS OF ELECTROCARDIOGRAMS OF CASE 2 (LEAD III)

Film No	Fig No	Date	Rate		Rhythm	Duration, Sec			Amplitude, Mm			Notes
			A	V		P R	QRS	Q T	P	S	T	
3277-1	6	4/25/21	77	77	Reg	0 19	0 10	0 31	0 75	7 0	0 5	Ventricular extra systoles Complete dissociation
3277-2	7	4/26/21	65	65	Irreg	0 31 to 0 12	0 10	0 38	-1 0	11 0	1 5	
3277-3		4/26/21	58	58	Reg		0 10	0 36	-1 0	7 0	1 0	
3277-4		4/26/21	56	57	Reg		0 10	0 36	-1 0	7 5	1 0	
3277-5		4/26/21	58	59	Irreg		0 10	0 36	-1 0	7 0	1 0	
3277-6	8	4/26/21	56	60	Irreg		0 10	0 37	-1 0	7 5	1 0	Series of V E S
3277-7		4/26/21	57	73	Irreg		0 11	0 38	-1 0	7 0	1 0	
3277-8	9	4/26/21	56	58	Irreg		0 11	0 38	-1 0	7 0	1 0	Auricular fibrillation, series V E S
3277-9		4/26/21	55	61	Irreg		0 11	0 38	-1 0	7 5	1 0	Series V E S
3277-10		4/26/21	56	64	Irreg		0 11	0 38	-1 0	7 5	1 0	
3277-11	10	4/26/21	50	48	Irreg		0 11	0 40	-1 0	7 0	0 5	Series of 46 V E S
3277-12		4/26/21	50	32	Irreg		0 11	0 44	-0 5	7 0	0 5	
3277-13		4/26/21	44	32	Irreg		0 11	0 42	-0 5	6 0	0 5	Auricular fibrillation
3277-14		4/26/21	?	55	Irreg		0 11	0 42	?	6 5	0 25	Auricular fibrillation, series of 51 V E S
3277-15		4/26/21	38	75 10	Irreg		0 11	0 41	?	5 0	0 25	Auricular fibrillation

Physical Examination—Emaciation. Pallor. Edema of dependent parts. Rales at bases of lungs. The relative cardiac dullness extended 12 cm to the left and 4.5 cm to the right of the midline. The pulse showed numerous extrasystoles with a deficit of 10 per minute. The liver edge was 4 cm below the costal margin.

Laboratory Findings—The blood showed a marked secondary anemia and a slight neutrophilic polymorphonuclear leukocytosis. Phenolsulphonephthalein, 59 per cent in two hours. Wassermann reaction negative. The urine showed albumin and a few hyaline and granular casts.

Course—The patient sank gradually and died April 26, three days after admission.

Necropsy Findings—Marked emaciation. Extreme anasarca. The heart weighed 720 gm. The coronary arteries were tortuous and sclerotic. The left ventricle was much hypertrophied and dilated, and contained a large mural thrombus measuring 7 by 5 by 5 cm. Mural thrombi were also present in both auricles. The myocardium showed numerous opaque grayish areas of scarring. Chronic passive congestion of the lungs. Multiple cysts containing concretions in the left kidney. Slight chronic diffuse nephritis. Generalized arteriosclerosis.

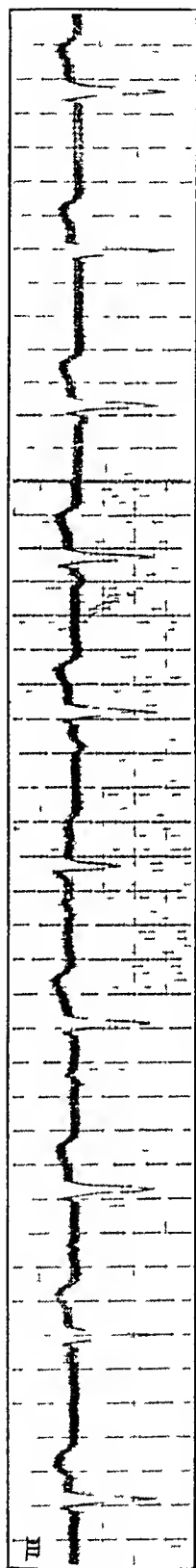


Fig 7—Case 2 Lead III April 26 Twenty minutes before patient died Note the changes in the P wave

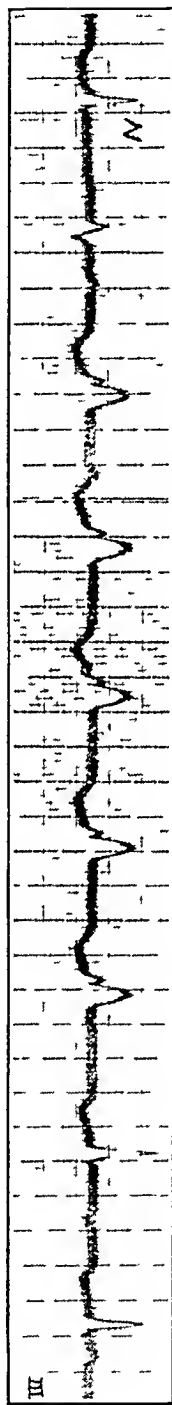


Fig 8—Case 2 Lead III Five minutes later Series of five ventricular extrasystoles with retrograde P waves

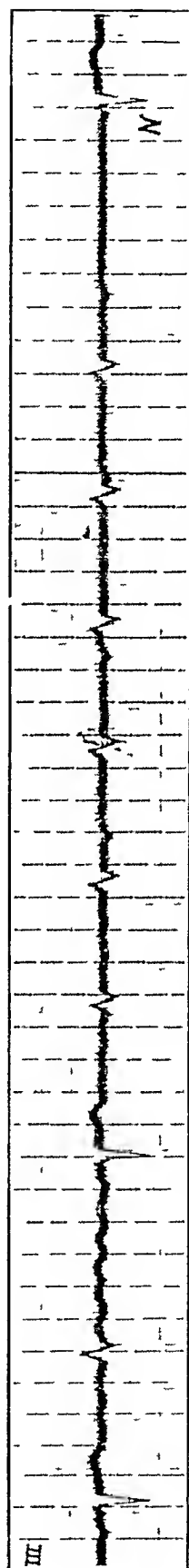


Fig 9—Case 2 Lead III A few minutes later than Figure 8 Transient auricular fibrillation and series of six ventricular extrasystoles of a second type N = normal complex

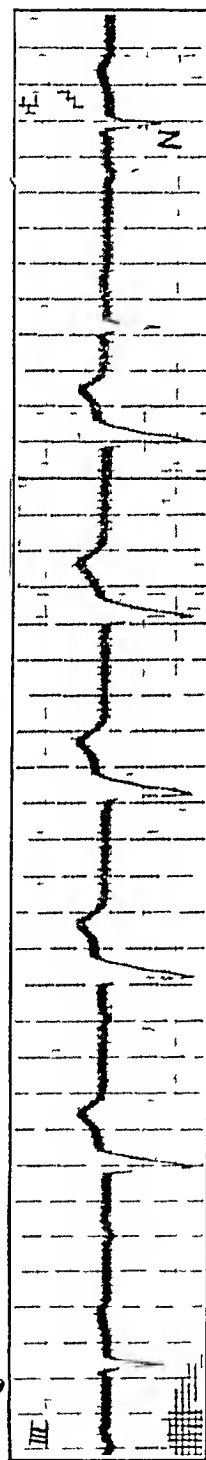


Fig 10—Case 2 Lead III Five minutes after Figure 9 Series of five ventricular extrasystoles of third type

Electrocardiographic Records—Electrocardiographic records of this patient were made April 25, two days after his admission, and again April 26, at short intervals during the thirty minutes just before and during the patient's death. Table 2 gives the measurements of these records (the figures are for Lead III throughout). The first record (Fig 6) shows a normal mechanism with left ventricular preponderance and occasional ventricular extrasystoles of a form which frequently recurred in the records of exitus. During the taking of Lead III of the second record (Fig 7) the P waves changed their form from positive to negative and a complete dissociation was apparently established. Occasional ventricular extrasystoles, mainly of three types, occurred throughout the remainder of the records. It is interesting that paroxysms or series of varying lengths of each form are found. A brief series of five ventricular extrasystoles of the first type with apparently retrograde P waves is shown in Figure 8, and records 14 and 15 show a series of fifty-six extrasystoles of the same form. Figure 9 is an illustration of the second type of extrasystole which is not retrograde but is accompanied by independent P waves and a brief period of auricular fibrillation. A short paroxysm of ventricular extrasystoles of a third form appears in Figure 10. This record just precedes the onset of a series of forty-six extrasystoles of the same type. This form also is accompanied by independent P waves at a slower rate. These series seem to meet the criteria suggested by Robinson and Herrmann¹³ for ventricular tachycardia, although in this instance the rate is only relatively rapid. There are four periods of temporary auricular fibrillation, one of which is mentioned above. Ventricular fibrillation did not occur in this case. The rate of the auricular contractions was established at from 56 to 58 at the onset of complete dissociation, and gradually fell to 38. The ventricular rate varied greatly from 70 to intervals in the last record corresponding to a rate of 10. The amplitude of R and T gradually decreased and the Q R S and Q-T intervals increased slightly. No tendency for R and T to fuse is shown. It would seem that the ventricles of this patient had several irritable foci which gave rise to the various abnormal types of ventricular complexes. It is interesting that notwithstanding this fact, ventricular fibrillation did not supervene.

The necropsy of this case revealed marked myocardial changes and abnormal left ventricular preponderance, thus confirming in a general way the important electrocardiographic findings.

CASE 3—M. M., female, white, aged 23, admitted April 27, 1921.

Complaint—Chills and fever, shortness of breath and palpitation.

¹³ Robinson, G. C., and Herrmann, G. R. Paroxysmal Tachycardia of Ventricular Origin, *Heart* 8 59, 1921.

Past History—Acute rheumatic fever at 12 Admitted to this hospital in 1911 with mitral stenosis and insufficiency, aortic insufficiency, bronchopneumonia and pleurisy After an interval, tonsillectomy was performed in the same year In 1919, the patient was on the obstetric service suffering from toxemia of pregnancy The patient had frequent sore throats, more or less constant slight dyspnea and transient slight edema of the legs

Present Illness—Onset two weeks before admission with sore throat, chills and fever The patient developed acute rheumatism She became very weak



Fig 11—Case 3 April 29, 1921 Second degree heart block Note the form of the Q R S complex

Physical Examination—Temperature, 101.6 F, pulse, 112, respiration, 28 Orthopnea and cyanosis were marked There were no râles in the lungs There was a marked precordial heave The apical impulse was 17 cm to the left of the midline The relative cardiac dulness measured 20.5 by 4.5 cm There was retromanubrial dulness, 3 cm to each side of the midline There was a loud systolic murmur at the apex and a "to and fro" murmur at the aortic area "Pistol shot" sounds were audible over the femoral vessels The pulse

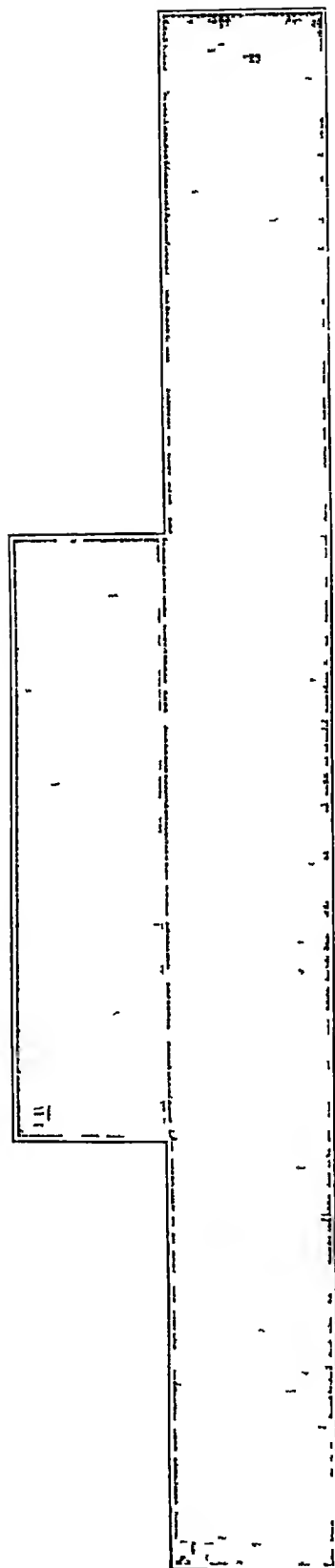


Fig 12—Case 3 Lead III May 3 Five hours before patient's death Complete heart block (see upper record) with onset of ventricular tachycardia

was regular and was of the "water hammer" type. There was a distinct capillary pulse. The blood pressure was 146/44. The liver edge was 5 cm below the costal margin and was tender. There was slight edema of the legs.

Laboratory Findings—The blood showed a secondary anemia and a slight neutrophilic polymorphonuclear leukocytosis, culture negative. The Wassermann reaction was negative.

Course—The patient received 18 cc of tincture of digitalis which was discontinued because of the occurrence of dropped beats. Three days later the pulse rate suddenly dropped to 38 and a Stokes-Adams attack occurred. This was repeated several times. One-fortieth grain of atropin was given with no effect on the pulse rate. Electrocardiographic records showed the presence of complete heart block with periods of ventricular tachycardia. The patient became progressively worse, with increasing dyspnea, cyanosis, pulmonary congestion and insufficient cerebral circulation. She was given 0.15 mg ouabain but grew weaker and died the same evening (May 2). There was no necropsy.

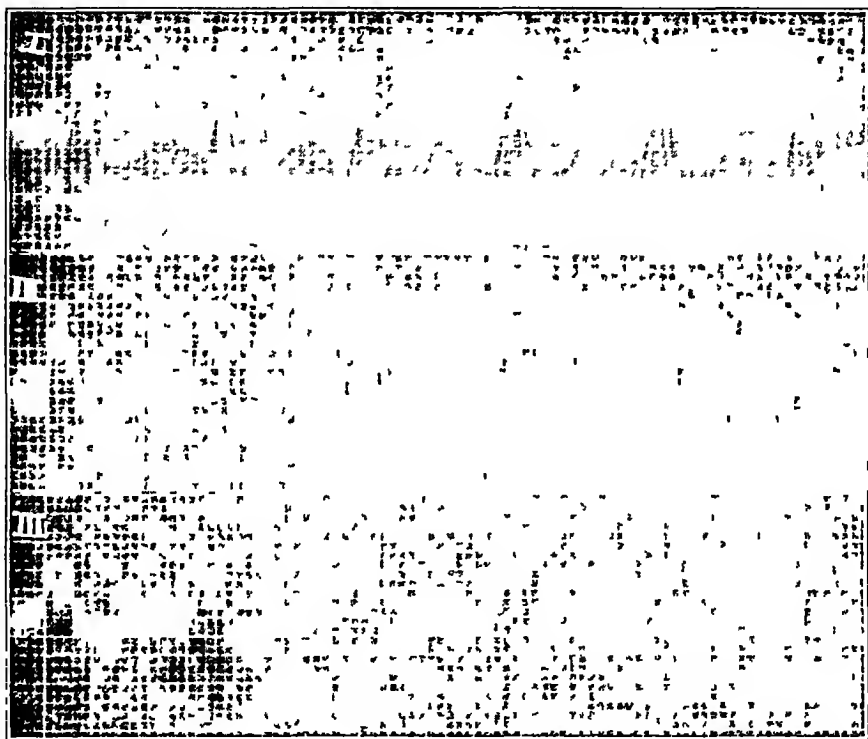


Fig 13—Case 3. A few minutes later than Figure 12. Ventricular tachycardia with retrograde P wave.

Electrocardiographic Records—An electrocardiographic record of this patient was made April 29, two days after her admission. It shows a long P-R interval which progressively increases until a beat is dropped and negative T waves (Fig 11). In view of the patient's history and of the small amount of digitalis administered and of the failure of atropin to relieve the block which later occurred, it is thought that these abnormalities indicate definite myocardial disease and that they were not the result of digitalis therapy. A second record was made May 3, about three hours before the patient died. This shows complete heart block interrupted by paroxysms of ventricular

tachycardia (Fig 12) The three leads of this tachycardia are shown in Figure 13 The form of the ventricular complexes in the paroxysms is entirely different from that which occurred during the complete dissociation In Lead I the ventricular complexes are followed by positive P waves (note that the P waves during the idio-ventricular rhythm are negative) Records were not obtained later

DISCUSSION

The electrocardiographic findings in these cases are to a certain extent fundamentally similar, in spite of the fact that it seems at first as if we were dealing with a heterogeneous group of records This is perhaps to be expected since in any terminal record there are not only the direct effects of the special cardiac lesions which may be present but also the results of the general pathological conditions which accompany extreme cardiac failure Marked impairment of the respiratory function with rapidly increasing insufficiency of oxygen and accumulation of carbon dioxide is the outstanding pathological condition from the functional point of view

The following features are common to the three cases described gradual slowing of rate, accompanied by lengthening of the P-R, Q R S and Q-T intervals and diminution in the amplitude of the R and T waves The normal pacemaker lost control of the rhythm in the first and second cases, probably due to failure both of stimulus production and of impulse conduction In these instances auriculoventricular nodal rhythm was established The P waves show that the auricles were contracting irregularly but independently, so that the block must have been retrograde as well as forward The independent auricular rate was slower than the ventricular and the auricular activity ceased before the nodal rhythm failed In the second case brief periods of auricular fibrillation occurred The sino-auricular node early ceased to be the pacemaker in the third case through the failure of the conduction system which was undoubtedly diseased in this instance This patient's clinical condition showed extreme anoxemia, the myocardium at no time being equal to the task of maintaining a proper circulation

The more individual abnormalities are, in the first case, interruption of the nodal rhythm by two periods of ventricular fibrillation In the second case a similar interruption by series of varying lengths of ventricular extrasystoles arising in at least three different foci Definite paroxysms of ventricular tachycardia occurred in the third case The underlying pathological physiology of these arrhythmias is probably fundamentally the same

The later records of the first case show to some extent a tendency for the R and T waves to coalesce a change which as was stated above the records of Robinson's ⁹ cases show consistently This phenomenon

is said by Samojloff¹⁴ to be associated with a lesion of the apical portion of the myocardium

In a recent contribution dealing with circulatory responses to oxygen want under experimental conditions Greene and Gilbert¹⁵ present data which are strikingly similar to the early general changes in the cases under discussion. There seems to be no doubt that the sino-auricular node is peculiarly susceptible to oxygen want and loses the function of impulse production early. The function of conduction is also greatly depressed. Whether this is due to vagospasm or direct myocardial asphyxiation is discussed by Greene and Gilbert without reaching any conclusion. No further light can be added on this question at present. Experimental work has not yet been carried to the point of ventricular arrhythmias such as occurred later in the cases presented. It would seem that it might be predicted that these arrhythmias will be found to develop when experimental data are gathered on the further effect of oxygen want after interference with impulse production in the auriculoventricular node. It should be pointed out that the present cases are complicated in comparison with those of Greene and Gilbert by the accumulation of carbon dioxid

SUMMARY

Terminal electrocardiographic records of three cases are presented and briefly discussed. The general changes which occurred were a gradual slowing of the cardiac rate with coincident lengthening of the P-R, Q R S and Q-T intervals and diminution in the amplitude of the R and T waves, and loss of control of the rhythm by the normal pacemaker, apparently with the inception of auriculoventricular nodal rhythm. Further, as a result of the functioning of various abnormally irritable foci, the following arrhythmias were observed in addition to heart block and nodal rhythm, auricular and ventricular extrasystoles, ventricular tachycardia and auricular and ventricular extrasystoles, ventricular tachycardia and auricular and ventricular fibrillation. It is probable that oxygen want, and perhaps carbon dioxid accumulation, following cardiac failure, underlie these abnormalities in such cases as those presented.

14 Samojloff, A. Weitere Beiträge zur Elektrophysiologie des Herzens, Arch f d ges Physiol **135** 417, 1910

15 Greene, C W, and Gilbert, A C. Responses of Circulation to Low Oxygen Tension, Arch Int Med **27** 517 (April) 1921

EXPERIMENTAL INQUIRY INTO THE CEREBRAL AND NEUROMUSCULAR MANIFESTATIONS OF DIGITALIS ¹

D I MACHT, M D AND WM BLOOM
BALTIMORE

INTRODUCTION

Cerebral symptoms in the course of heart disease are not at all uncommon. Everyone is familiar with the classical work of Head,¹ and several other interesting publications on the subject such as by Rieseman² and others. There is, however, one group of psychoses occurring in cardiac patients which has perhaps received insufficient attention on the part of clinicians. These are the patients exhibiting hallucinations, delirium and other mental affections occurring at the height of digitalis therapy. The father of rational digitalis therapeutics, Withering,³ did not fail to note certain cerebral manifestations of digitalis and mentions them causally in his famous study of the foxglove.

"The foxglove when given in very large and quickly repeated doses occasions sickness, vomiting, purging, dizziness, distorted vision-objects appearing green or yellow, increased secretions of urine with frequent motions to part with it, slow pulse even as slow as 35 to a minute, cold sweats, convulsions, syncope and death" (p 184)

The most important contribution on this subject, however, came from an eminent French physician, Duroziez, in 1874⁴. He gives a list of twenty cases in which delirium or hallucinations, with or without death accompanied the administration of digitalis, and which he believed were caused thereby. H O Hall, in 1901, revived the interest in the subject. He reviewed the cases of Duroziez and others and described one from his own experience.

We have become interested in the question whether the digitalis group of drugs produce any cerebral effects in connection with the study of the action or influence of various drugs⁶ on the behavior of

* From the Pharmacological Laboratory, Johns Hopkins University

1 Head Brain **24** 345, 1901

2 Rieseman Tr Ass Am Phys **35** 77, 1920

3 Withering An Account of the Foxglove, Birmingham, 1785

4 Duroziez Gaz hebdom **11** 780, 1874

5 Hall Am Med **1** 598, 1901 *ibid* **9** 489, 1905

6 Macht and Mora J Pharmacol & Exper Therap **16** 219, 1920 Macht and Bloom *Ibid* **27** 21, 1921

white rats in the circular maze and we have performed a series of experiments with various bodies in order to clear up at least partially, this subject

DESCRIPTION OF THE MAZE

The circular maze shown in Figure 1 is made with wooden base and aluminum walls. The base is 15 cm in diameter and 4 cm in thickness. Its upper surface is marked off by grooves into a series of concentric circles. The diameter of each of the circles is as follows, beginning with outermost one: 140 cm, 120 cm, 100 cm, 60 cm, 40

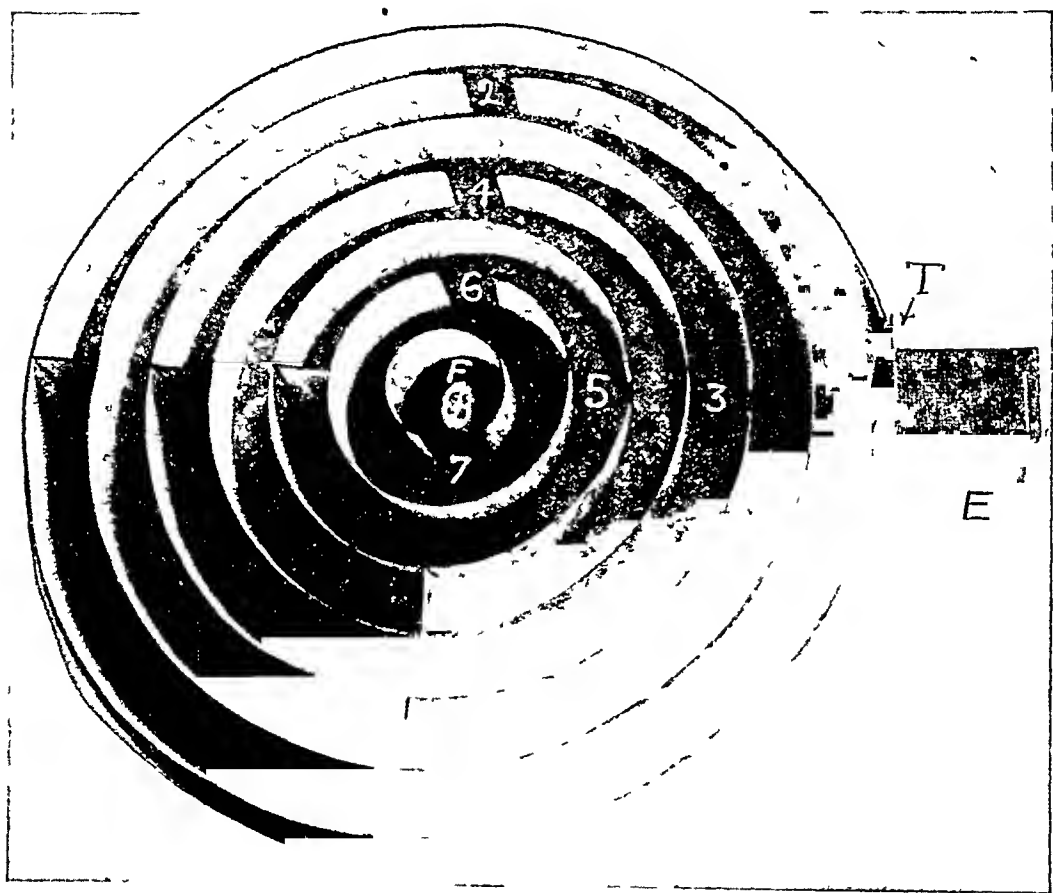


Fig 1—The circular maze used to study drug action

cm and 20 cm. Into the circular grooves are inserted sheets of aluminum 18.5 cm high and 0.8 mm thick. Each strip of aluminum is cut 10 cm shorter than the length of the circular groove into which it is to be fitted, thus giving an opening into the alley. By means of this arrangement it is possible to slide the aluminum around in its groove and thus to place the entrance in any desirable position. In the present investigation the openings or entrances to the alleys were placed in the position indicated in Figure 1, there being seven openings so arranged that the rat had to make alternate turns to right and left,

in the order indicated by Nos 1 to 7 In addition to the doors or openings, the alleys were also provided with obstructing partitions, which formed a number of blind cul-de-sacs

The camera lucida attachment, invented by Watson,⁷ is illustrated in Figure 2 A large plate-glass mirror M, 91 cm wide and 121 cm in

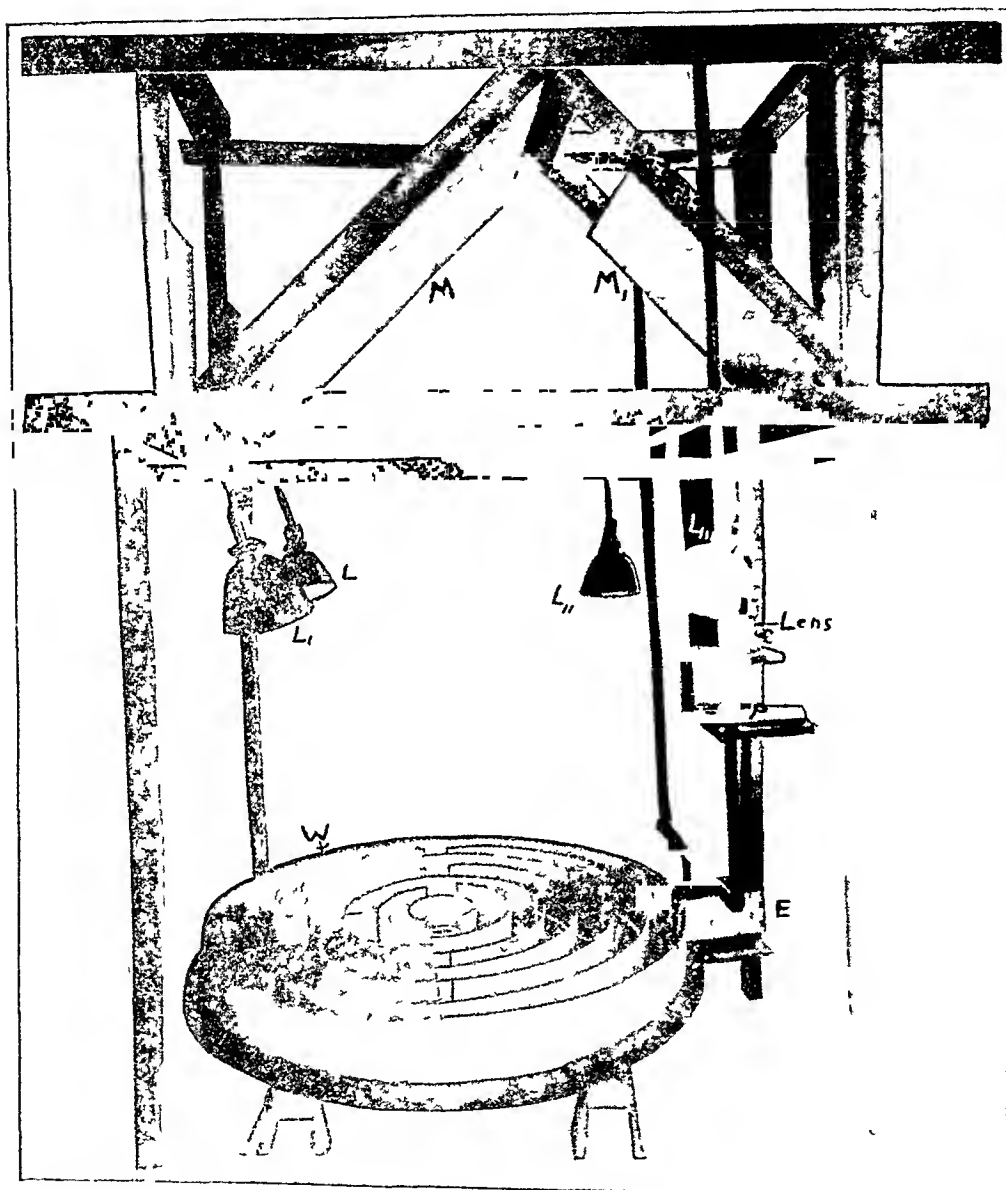


Fig 2—Camera lucida attachment used in connection with the circular maze

length, was placed at an angle of 45 degrees, directly over the maze, at such a distance from the first mirror that the light reflected downward from it falls outside the maze Below M, and in the light

⁷ Watson J *Animal Behavior* 4 56, 1914

reflected from it, is placed a single acromat, 6 cm in diameter and 50 cm focus. The lens is placed in a barrel and the barrel is attached to a wooden disc 30 cm in diameter. This board is attached to an iron collar which slides freely up and down and gives a very easy means of adjusting the size of the image. A pad of circular paper is laid on a wooden shelf, below the lens, and the distance is so adjusted that the reduced image of the maze is focused on the paper. Extraneous light is excluded by means of a soft, dark flannel curtain, not shown in the figure. As may readily be seen, the maze must be illuminated quite highly in order to produce a clear image. This illumination is obtained by means of four electric lamps, with opaque shades, placed symmetrically around the maze. By means of the camera lucida attachment, the movements of an animal in the maze can be traced on white paper with a soft pencil. Such tracings are especially useful in the study of the learning of the maze problem, such as has been done by Miss Hubbert and others. In the present investigation, where the effect of drugs on the behavior of the animals was studied after the rats had been trained, the use of this attachment was not essential and it was, therefore, dispensed with in many of the experiments.

The study of the behavior of the rats in the circular maze is begun by placing an animal in the center of the maze and feeding it from the bowl F for three successive days. During these three preliminary feedings, which last from ten to fifteen minutes, entrance 7 is blocked off, so that the animal may not roam around. On the fourth day, the rat is placed in the cage E, then the trap-door T is raised and the animal allowed to enter the first alley. The animal then gradually learns to find its way to the center of the maze, when it is taken out and the experiment is repeated. Generally, three trials are made on each day. For work with the maze, albino rats, which are very tame, must be employed. The animals must be handled gently with the hands, and under no circumstances must they be picked up with forceps or similar instruments. The most suitable animals are found to be rats approximately from 60 to 90 days old. Older animals are apt to be sluggish, while very young rats do not learn the maze problem so readily. Ordinarily, the albino rats learn the maze problem in about two weeks, and sometimes within a shorter period of time. An animal is considered to have the maze problem when it has learned to find its way into the center of the maze by the shortest route, that is, without any errors, on three successive trials. The technic of the training is described more in detail by Hubbert.⁸

⁸ Hubbert J. *Animal Behavior* 4 60, 1914

ANALYSIS OF THE DATA FURNISHED BY THE MAZE

The maze problem enables the psychologist to study the mode of learning of a rat. In studying the effect of drugs, the maze problem can be utilized in two ways. Animals may be subjected to the influence of drug action first and then trained in the maze with the purpose of ascertaining the effect on the rate of learning. Again, animals may be first taught to solve the maze problem and then the effect of drugs is studied in reference to its influence on their behavior, memory-habit, etc. Furthermore, other data can be obtained from the maze, after administering drugs to rats, which may show the effect on neuromuscular coordination, and various somatic changes. As to exactly what the mechanism of learning the maze problem may be, the explanations given by various psychologists differ widely. Among the hypotheses which have been advanced to account for the reintegration of conduction paths in learning, there are at least three which stand out as rather opposed to one another in respect to the neural processes which they imply.⁹ The hypothesis suggested by Ladd and Woodworth¹⁰ assumes the inhibition of successive activities as the fundamental process which results in the selection and fixation of random activities. The second hypothesis, such as given by Angell,¹¹ assumes nervous reinforcement as the fundamental process by which successive acts become linked together in habit formation. The third hypothesis (Watson) depends chiefly on the chance spreading of nervous excitation, or the simultaneous activation of two afferent pathways, in such a way that the final common part of one is able to divert the discharge of the other, and so bring about a permanent connection between itself and this afferent path.¹² These hypotheses by no means exhaust the theoretical considerations of the maze problem (Dashiell¹³). For the study of drug action, however, the various theoretical considerations are of secondary importance and the data obtained are of a much more definite nature, as will be seen from the following exposition.

EXPERIMENTS WITH DIGITALIS BODIES

In the present investigation about forty rats were used. The animals weighed on an average 150 gm each and were for the most part males, a few females, however, were also trained and studied. The animals were taught to solve the maze problem and after having learned to run to the center as quickly as possible and without making

9 Lashley *Psychobiology* 1 141, 1917

10 Ladd and Woodworth *Elements of Physiological Psychology*, 1911, Scribner's Sons, New York, p 551

11 Angell *Psychology*, Ed 4, Holt, New York, 1909, p 70

12 Watson *Behavior: An Introduction to Comparative Psychology*, Holt, New York, 1914

13 Dashiell *Psychobiology* 2 4, 1920

any mistakes they were ready for experimentation. In every experiment three normal trials were first made, then the animals were injected intraperitoneally and sometimes intramuscularly with the drugs selected and at the expiration of one half hour the behavior in the maze was again studied. In many experiments a second observation of three trials each was made several hours after the injection in order to note the later manifestations of the drugs, and, furthermore, the behavior of the rats was observed on the subsequent days until the animals either died or as was usually the case after small doses, completely recovered.

The following drugs were studied: ouabain (or crystalline strophanthin), Merck's amorphous strophanthin (Kombé), digitoxin, digitonin, various samples of digitalin, digalen, and several injectable preparations of digitalis leaf, such as digitotal, digitupuratum and digitofoln. Lastly, a few experiments were also made with the interesting heart drug related to the digitaloids which was isolated by Abel from the skin secretion of the toad and was named bufagin¹⁴.

The results of most of the experiments are shown in Table 1. As will be noted, all of the drugs studied, when given in sufficient quantity, produced depression in the rats. This was indicated by the failure in discrimination and memory-habit as expressed by the number of errors made in the maze. Furthermore, the drugs distinctly slowed the running speed of the animals and produced disturbances in neuromuscular coordination and general behavior. Large doses of the digitalis drugs produced a marked paresis or weakening of the hind limbs as well as a general narcotic condition. But even small doses as will be noted from the table produced a distinctly depressant effect. Thus it will be seen that as little as 0.2 mg. of ouabain and 0.15 mg. of amorphous strophanthin markedly depressed the animals. Such doses cannot be regarded as near the fatal limit of the poisons, inasmuch as it is well known that rats are very resistant to strophanthin. In this connection, it may be well to state that we have confirmed a remarkable observation made by Hatcher some years ago¹⁵ and found that in case of the rats amorphous strophanthin is more toxic than ouabain, which is contrary to the findings in regard to the relative toxicity of these two poisons for other animals. We have found the lethal dose of amorphous strophanthin for rats to be about 1 mg. per 40 gm. weight of the animal, which dose generally produced death within an hour. On the other hand, the lethal dose of ouabain is 1 mg. per 30 grams of weight.

14 Abel and Macht *J. Pharmacol. & Exper. Therap.* **3**: 319, 1911.

15 Hatcher *Am. J. Physiol.* **23**: 303, 1909.

TABLE 1—RESULTS OF EXPERIMENTS

Experiment	Drug	Dose per 150 Gm Weight	Before Injection		Half Hour After Injection		Four Hours After Injection		Effect	Late Effect
			Time	Errors	Time	Errors	Time	Errors		
1	Ouabain Sol	0.01 mg	21	0	16	0	15	0	None	None
2	Ouabain Sol	0.10	13	0	15	0	17	0	None	None
3	Ouabain Sol	0.20	13	0	14	0	14	0	None	None
4	Ouabain Sol	0.20	14	0	12	0	18	0	Slight depression	None
5	Ouabain Sol	0.20	45	2	75	3	53	0	Depression, jerky movements	None
6	Ouabain Sol	0.50	18	0	73	2	29	1	Great depression	Depressed next day
7	Ouabain Sol	1.00	21	0	51	2	22	0	Depression	
8	Ouabain Non Sol ampule	0.25	13	0	165	5	22	0	Depression	
9	Ouabain soft glass ampule	0.25 (?)	18	2	42	0	18	0	No effect	
10	Ouabain Sol	5.00							Death, 1 hour	Depressed 2 days
11	Strophanthin amorphous (Merek)	1.00	26	0	Paralysis		Paralysis		Death, 1 hour	Slight depression
12	Strophanthin amorphous (Merek)	4.00	18	0	Stalled				Depressed 2 days	Slight depression
13	Strophanthin amorphous (Merek)	0.15	32	0		7			Depression	Slight depression
14	Strophanthin amorphous (Merek)	0.25	50	0	89	4			Depression	Slight depression
15	Strophanthin amorphous (Merek)	0.15	26	2	107	5			Depression	Slight depression
16	Strophanthin amorphous (Merek)	0.20	22	0	66				Great depression	Depression
17	Strophanthin amorphous (Merek)	2.00	21	0	Hind limbs paralyzed					
18	Digitalin, German	1.00	25	0	19	0	18	0	None	Depression 2 days
19	Digitalin, German	2.00	32	0	84	2	70	0	Marked depression	
20	Digitalin, Kiliani	1.00	25	1	27	1	24	0	Slight depression	
21	Digitalin, Kiliani	2.00	23	0	78	3	58	0	Depression	
22	Digitonin	0.20	32	0	28	0	27	0	None	Recovery
23	Digitonin	1.00	32	0	23	0	14	0	Slight narcosis	Recovery
24	Digitonin	1.00	13	0	Narcosis	0	14	0	Depression	Recovery
25	Digitonin	2.00	15	0	Completely lost	0	14	0	Prostration, depression and cyanosis	Paralysis next day
26	Digitoxin	0.50	59	3	lost	1	Stalled	2	Marked depression	Paralysis next day
27	Digitoxin	0.50	44	0	44	0	31		Marked depression	Recovery
28	Digitalin	1 ampule	14	0	14	0			Depression	Recovery
29	Digitalin	1 ampule	15	0	Stalled	0	33	1	Slight depression	None
30	Digipuratium	1 ampule	29	0	26	0	16	0	Slight depression	None
31	Digifolin	1 ampule	16	0	27	0	36	0	Depression	None
32	Digifolin	1 ampule	32	0	Stalled	0	29	0	No effect	None
			31	0	27	0				

DISCUSSION

The results of the above described experiments indicate that the various members of the digitalis group of drugs produce a depressant effect on the behavior of rats in a maze, as shown by their loss of memory, by their slower gait, and by neuromuscular incoordination. Whatever theory one may hold in regard to the psychologic data furnished by the maze problem, it will be generally conceded that the effects produced by the drugs must be referred for the most part to the central nervous system and at least to some extent, to the higher centers of the same. The data obtained from the rats, therefore,

TABLE 2—SUMMARY OF DUROZIEZ' CASES

No of Cases	Form of Drug	Dose	No of Doses	Result
1	Alcoholic extract of digitalis	30 to 40 cg	6 doses	Delirium
2	Alcoholic extract of digitalis in soup	40 cg	2 doses	Delirium and death
3	Digitalin	20 to 30 cg	18 doses	Delirium
	Powder of dig	4 to 5 mg	1 month	Delirium
	Powder of dig	30 cg	1 month	Delirium
	Powder of dig	30 to 40 cg	1 month	Delirium and death
4	Powder of dig	20 cg	42 days	Delirium and death
5	Dig in maceration	1 gm	4 days	Delirium
6	Dig in maceration	1 gm	1 day	Delirium
	Dig in maceration	1 gm	4 days	Delirium and death
7	Dig in maceration	1 gm	2 doses	Hallucination
8	Alcoholic tincture	1, 2, 3 and 4 gm	20 doses	Delirium and death
9	Alcoholic tincture	40 gm	4 days	Delirium
10	Wine of Trousseau	27 gm	9 days	Delirium and death
11	Dig in maceration	15, 50 and 60 cg	17 days	Delirium and death
12	Dig in soup	1 gm and 1 m , 50 cg	4 days	Delirium and death
13	Dig in soup	20, 40 and 60 cg	3 days	Delirium and death
14	Dig in soup	40, 60 and 30 cg 60 and 80	3 days	Delirium and death
15	Dig in soup	1 gm , 1 gm	4 days	Delirium and death
16	Dig in soup	1 gm		Sudden death
17	Dig in soup	30, 40 and 50 cg	10 days	Delirium and death
18	Dig in soup	1 gm	3 days	Delirium and death
19	Dig in soup	15 and 20 cg	13 days	Delirium and death
20	Digitalin and wine of Trousseau	1 gm and 50 cg		Delirium and death

would seem to confirm the clinical observations of Duroziez and other authors. Table 2 shows a summary of Duroziez' cases. We are inclined to think that such cases are more common than is generally supposed, for at least three prominent physicians who were questioned on the subject replied that they have personally observed such cases, and Dr A D Hirschfelder, in a personal communication to one of us, described a case in which the patient, a man suffering with heart trouble, was made suddenly delirious and demented by full doses of digitalis, and while in that state of mental aberration, committed homicide. That patient subsequently recovered his mentality and at the court trial was acquitted, the plea of the defense being that he committed the crime while in a state of dementia produced by digitalis. It is hoped that the present communication will arouse the interest of the clinician in the subject.

SUMMARY

1 The effects of various digitalis bodies were studied on the behavior of rats in the circular maze

2 It was found that the various digitaloids even when given in comparatively small doses produced a depression in the behavior of animals

3 These experimental observations agree with the clinical experiences of Duroziez and other clinicians

Archives of Internal Medicine

VOL 28

DECEMBER, 1921

No 6

THE BLOOD IN TETRACHLORETHANE POISONING*

GEORGE R MINOT, MD, AND LAWRENCE W SMITH, MD
BOSTON

Tetrachlorethane is a substance that readily poisons man, causing a fatal toxic hepatitis when it enters the body in sufficient amounts. It is used commercially because of its peculiar physical and chemical properties. It is a solvent for cellulose acetate and is both waterproof and noninflammable. Tetrachlorethane has risen to a point of paramount interest in the last few years because of its use in some of the so-called aeroplane "dopes"—the varnish with which the linen wings of aeroplanes are painted.

During the war, numerous cases of poisoning from use of this substance were reported among those engaged in the manufacture of aeroplanes in England, America and Germany. Some deaths occurred from this substance in England and Germany but none have been reported from America. Tetrachlorethane is being utilized in this country in the manufacture of such articles as noninflammable films, various lacquered goods and artificial silk.

The president of an artificial silk plant, employing about sixty persons at one time, requested one of us to supervise for five months an investigation of the health of his employees, aiming particularly to learn how to prevent any serious form of tetrachlorethane poisoning. The general clinical aspects of this investigation have been conducted by Dr Derric C Parmenter¹. The part of this general investigation that is reported here was undertaken by us to determine whether tetrachlorethane produced any abnormality of the blood, and, if so, whether mild poisoning could be detected or even anticipated by blood examination. It was hoped that a blood examination might be of value in aiding the regulation of employment in such a plant.

* From the Medical Laboratories of the Massachusetts General Hospital.

* This is paper No 2 of a series of studies on the physiology and pathology of the blood from the Harvard Medical School and allied hospitals, a part of the expense of which has been defrayed from a grant from the Proctor Fund of the Harvard Medical School, for the Study of Chronic Disease.

* Presented in abstract before the Association of American Physicians, Atlantic City, N J, May 11, 1921.

1 Parmenter, D C J Indust Hygiene 2 456 (April) 1921.

Because tetrachlorethane may produce a toxic hepatitis somewhat similar to that which may be produced by arsenobenzol, arsphenamin, etc., it was thought that the blood of tetrachlorethane workers might show increases of large mononuclear cells, as Evans² and one of us has observed occasionally occurs with the initial stages of poisoning from these arsenic products. It was also thought that blood changes in the nature of destruction of red cells, with increased marrow activity, might occur like those due to trinitrotoluene,³ dinitrophenol and dinitrobenzene, substances which produce a toxic hepatitis.

Before discussing the blood findings in the sixty-eight persons we have studied, who were exposed and some who were poisoned by tetrachlorethane, it is desirable to summarize briefly the symptomatology and pathology of tetrachlorethane poisoning.

From the literature, which is chiefly made up of reports by Wilcox⁴ and others,⁵ a summary by Hamilton,⁶ and by reports from Jungfer,⁷ Grimm, Heffter and Joachimoglu,⁸ and Koelsch,⁹ we obtained a fairly good clinical picture of particularly the severer tetrachlorethane poisoning.

The subjects of our study have given us a clearer picture of the early symptomatology of this form of industrial poison than can be obtained from the literature.

The earlier symptoms consist of three chief types, general, gastric and nervous. In a typical case, the onset is marked by a sense of

2 Evans, F. A. Observations on the Origin and Status of the So-Called "Transitional" White Blood Cell, *Arch Int Med* **17** 1 (Jan) 1916.

3 Minot, G. R. Blood Examinations of Trinitrotoluene Workers, *J Indust Hygiene* **1** 301, 1919.

4 Wilcox, W. H. A Fatal Case of Poisoning by Tetrachlorid of Ethane, *Lancet* **2** 1489, 1914. An Outbreak of Toxic Jaundice Due to Tetrachlorethane Poisoning. A New Type Among Aeroplane Workers, **1** 544, 1915. The Treatment of Toxic Jaundice due to Tetrachlorethane Poisoning, *Brit M J* **1** 300, 1916.

5 Legge, T. M. Origin, Symptoms, Pathology, Treatment and Prophylaxis of Toxic Jaundice Observed in Munition Workers, *Proc Roy Soc Med (Sect Med, Path & Epidemiol)*, January, 1917, p. 2. Spilsbury, B. H. Origin, Symptoms, Pathology, Treatment and Prophylaxis of Toxic Jaundice Observed in Munition Workers, *Proc Roy S M (Sect Med, Path and Epidemiol)*, January, 1917, p. 42. Lee, W. E. A Fatal Case of "Dope Poisoning," *Lancet*, **1** 24, 1916. Barlow, F. Dope Poisoning, *Med Press & Cir* **152** 471, 1916.

6 Hamilton, A. Industrial Poisoning in Aircraft Manufacture, *J A M A* **69** 2037 (Dec 15) 1917.

7 Jungfer. Tetrachlorathanvergiftungen in Flugzeugfabriken, *Zentralbl f Gewerbehyg* **2** 222, 1914.

8 Grimm, V. Heffter, A., and Joachimoglu, G. Gewerbliche Vergiftungen in Flugzeugfabriken, *Vrtljschr f gerichtl Med*, 1914, Ser. 3, 48, Sec. Suppl., 162, 191.

9 Koelsch, F. Gewerbliche Vergiftungen durch Zelluloidlacke in der Flugzeugindustrie, *Munchen med Wchnschr* **62** 1567, 1915.

abnormal fatigue, the patient perspires freely, drowsiness develops, with loss of appetite, nausea, vomiting, constipation and headache. After days, or even weeks, jaundice develops, and the gastric and nervous symptoms increase. There then may be confusion, delirium, coma and death. The preliminary symptoms of abnormal fatigue, general discontent and nervousness, inability to concentrate, loss of appetite, headache and insomnia, constitute a clearly defined picture of the beginning of poisoning. These symptoms may develop over a period of several days, or over a period of a few weeks. The insidious onset of such rather minor symptoms is a distinct feature, and they serve as a warning of the severer symptoms which may follow unless the individual is removed from exposure to the poison. Owing to their insidious onset and mild character, these minor symptoms are readily overlooked.

Severer symptoms consist of an increase of constipation, nausea and vomiting, and, in addition, gas in the stomach, generalized abdominal pain, dizziness and very slight jaundice. Such disturbances may appear suddenly, but this is unusual. Still more advanced symptoms occurring with increase of jaundice are those of cholemia, which occur associated with severe necrosis of the liver cells, very similar to those of acute yellow atrophy. No other important pathologic change has been reported.

Besides jaundice, which may become moderate in degree, the important physical signs which develop relatively late, consist chiefly of an occasional palpable and tender liver, general abdominal tenderness, pallor and loss of weight.

Immediately following removal from exposure, the symptoms do not necessarily subside but not infrequently increase for a few days. If the symptoms are of the very mildest degree, they may disappear within two or three days, even without removal from exposure. Jaundice, even though not severe, when it is once established may persist for several weeks or even months before disappearing.

There were no severe cases of tetrachlorethane poisoning among the sixty-eight employees of the artificial silk plant whom we studied during a period of five months. Twenty of these were women and were practically not exposed to the poison. The others were men exposed to a greater or lesser degree at one time or another. Among them were twelve who showed symptoms best interpreted as very mild preliminary symptoms of poisoning which persisted for a few days only. Their symptoms consisted of an abnormal sense of fatigue, irritability, constipation and headache. There were nine others who showed more definite symptoms of poisoning. Two of these were moderately sick, but not to a degree to be considered serious or alarming, and not at any time sick enough to warrant their going to bed.

In all, 275 critical Wright stained blood smear examinations of the white cells, red cells and platelets were made on sixty-eight employees. Sixty white cell counts (thirty-seven persons) and forty-four red cell counts (thirty persons) and Tallquist hemoglobin determinations were made¹⁰. A few other observations on the blood were made but yielded no significant information.

The only reference we have found in the literature to the blood in tetrachlorethane poisoning is by Wilcox,⁴ who merely states that "the jaundice is hepatogenous, not hemolytic, and that no appreciable anemia or changes in the red blood corpuscles occur."

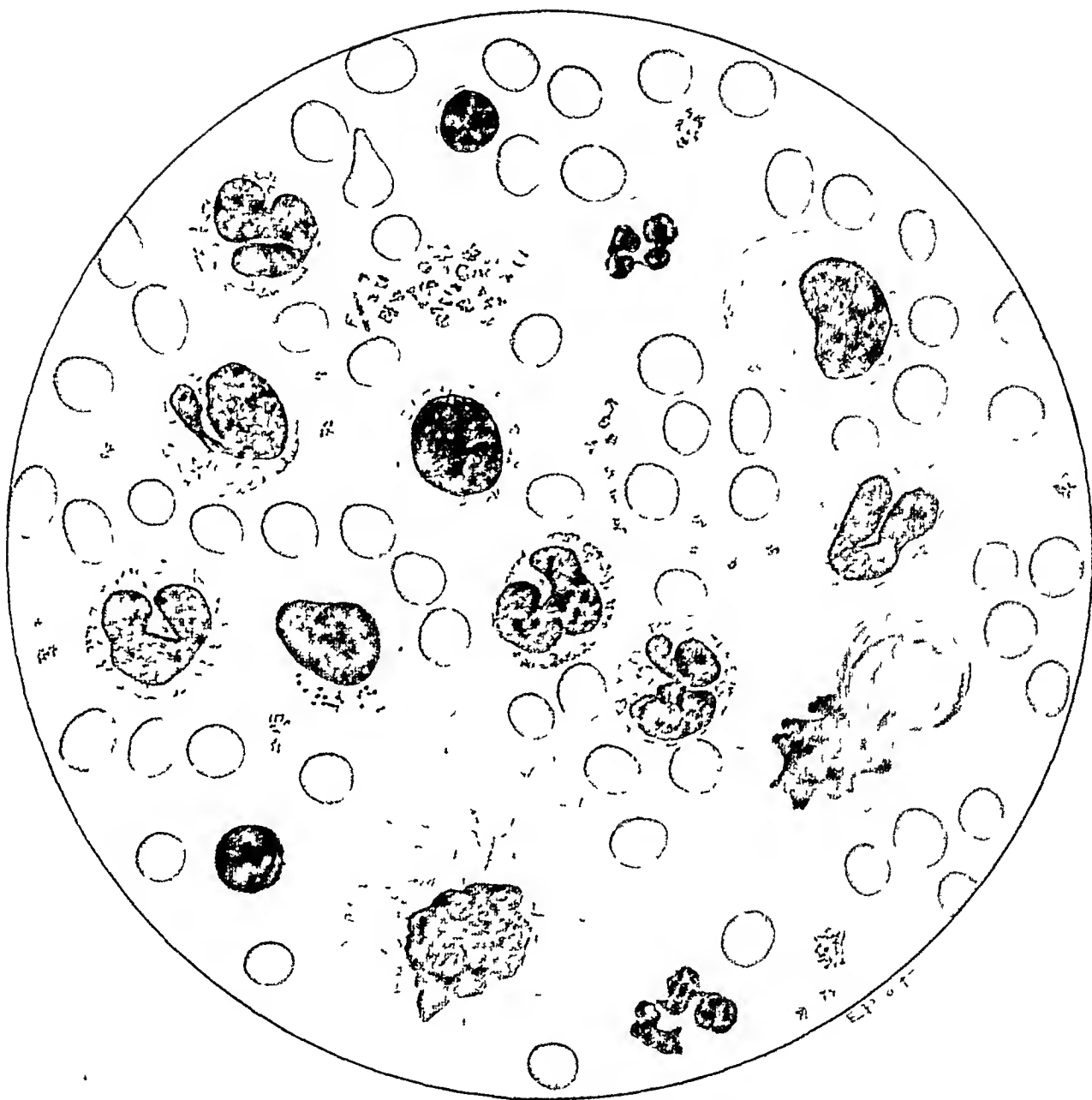
The blood of certain employees whom we have studied showed distinct abnormalities, the most important alteration from normal being an increase of the large mononuclear cells¹¹.

Nearly all of the twenty-five persons whose blood showed this change to a definite degree, had or soon developed, especially if there was a progressive increase of these cells, some symptoms attributable to poisoning. On the other hand, none of the twenty-one employees who had symptoms due to tetrachlorethane failed to show increases of the large mononuclear cells, which often formed over 30 per cent of the leukocytes. Other less marked and less significant changes have been found by us in the blood of persons poisoned by this chemical.

Table 1 consists of an analysis of a typical case of poisoning. It shows the progression of symptoms eventuating in jaundice which remained for several weeks after the other symptoms had disappeared. The table also shows that as the symptoms increase, progressive alterations in the blood develop, which returns toward normal as the symptoms subside. The blood changes, particularly, consist of the following features: (1) The progressive increase of the large mononuclears, (2) the progressive increase of young large mononuclears, some formed and some broken, (3) the somewhat elevated white count, (4) the slight but progressive anemia, and (5) the slight increase of platelets.

¹⁰ It is a pleasure to acknowledge the technical assistance given by Miss Margaret Weld.

¹¹ In this paper, the term large mononuclear cells (Mallory's endothelial leukocyte) refer to those classically twice as large as a polymorphonuclear neutrophil, which are often considered to originate in the bone marrow and from endothelium to which the inappropriate term of transitional cell has been applied, especially to the more mature form with the indented nucleus. These cells are very similar to those large mononuclear cells that probably originate in lymphatic tissue, but they are usually larger, more sharply defined, more granular and stain more distinctly than the large mononuclear cells of lymphatic origin. It is quite possible that owing to the liver pathology of tetrachlorethane poisoning that the large mononuclear cells in these poisoned individuals have come from the endothelium of the liver as well as from the marrow. Both Dr C H Bunting and Dr J H Wright have been kind enough to examine some of the preparations and agree that the cells are of the type referred to.



The blood of a case of tetrachlorethane poisoning, stained with Wright's stain. The white cells occurred in an actual field under 570 magnifications. The drawing was made by aid of a camera lucida under 1,425 magnifications. Seven large mononuclears are present, five mature ones (transitional cells) with indented nuclei and two immature, without definite indentation of their nuclei. The cell in the lower left hand quadrant with reddish granules in the protoplasm is an atypical large lymphocyte. Two normal small lymphocytes and two normal polymorphonuclear neutrophils are shown, and three broken white cells. The larger broken cell in the lower right hand quadrant is especially characteristic.

The typical blood picture is shown in the accompanying colored plate

All of the sixty-eight persons studied have been placed into three groups, as shown in Table 2, dependent, on the one hand, on the number of large mononuclear cells in the blood, and on the other on the symptoms that occur. In this table, the figures and data shown represent an average obtained at the time that the large mononuclear counts were the highest in each case

TABLE 1—ANALYSIS OF A TYPICAL CASE OF TETRACHLORETHANE POISONING

Date	W B C in Thousands	Per Cent of					Broken W B C per 100 Formed W B C	R B C in Millions	Red Cells *				Platelets †	Symptoms ‡
		Poly-norphonuclear neutrophils	Lymphocytes	Large Mononuclears	Eosinophils	Basophils			Achromia	Anisocytosis	Poikilocytosis	Polychromatophilia		
Jan 2	90	59	33	6	2	0	5	50	0	0	0	0	N	0
Feb 18	85	56	31	13	0	0	4	40	—	—	—	—	N	0
Apr 20	112	50	30	20	0	0	8	40	+	0	0	0	N	0
May 1	120	45	25	20	1	0	13	47	++	+	0	0	+	+
May 13	156	39	20	40	1	0	31	47	++	++	++	++	++	++§
May 16	152	43	22	35	0	0	30	47	++	++	++	++	++	++§
May 23	114	45	20	25	1	0	18	47	+	+	0	0	+	++§
June 6	106	50	38	12	0	0	7	47	+	+	0	0	+	0

* Red Cells +, indicative of earliest recognizable changes—very slight achromia, just distinguishable variation in size or shape and one polychromatophilic cell per 10-12 oil immersion fields, ++, moderate achromia, definite but slight variation in size and shape, one polychromatophilic cell per 45 oil immersion fields

† Platelets +, very slight increase in numbers, ++, moderate increase in numbers N, normal

‡ Symptoms degree indicated by + signs +, abnormal fatigue, discontent, constipation, headache, ++, a greater degree of above symptoms, coupled with insomnia and irritability, ++++, further increase of above symptoms with addition of anorexia, nausea, vomiting and slight jaundice

§ Taken off work

† Jaundice still present, no subjective symptoms

Group I consists of all employees, twenty-five in number, who showed an increase of large mononuclear cells above 12 per cent in a differential count of not less than 200 white cells. Twelve per cent has been chosen rather arbitrarily but chiefly because of the fact that 10 per cent of large mononuclears may occur in the absence of any clearly recognized pathology, and this number of these cells occurred in several people in the other groups. All of the twenty-five persons in Group I manifested clinical symptoms of poisoning at one time or another, except four. These four persons have been placed in a subgroup (labelled B in the table) and will be discussed later.

Group II consists of twenty-three male employees whose large mononuclear cells remained below 12 per cent. These men showed no symptoms of poisoning in spite of exposure to tetrachlorethane, to essentially the same degree as those placed in Group I.

In Group III are twenty women whose large mononuclear cells were below 12 per cent and who had no symptoms of poisoning. They serve fairly well as a control group as they had almost no exposure to the poison.

Groups II and III require no further comment as neither symptoms nor significant blood abnormalities occurred.

The pathologic increase of the large mononuclear cells, together with other blood changes which occurred in the cases of Group I, are to be interpreted critically. The typical blood changes that occurred in the subjects placed in this group have been shown in Table 1. The typical case shows the large mononuclears rising progressively from approximately 10 to 40 per cent over a period of from four to eight

TABLE 2—SUMMARY OF BLOOD FINDINGS IN SIXTY-EIGHT TETRACHLORETHANE WORKERS

	Number of Cases	W B C in Thousands†	Per Cent of					Broken W B C per 100 Formed W B C	R B C in Millions†	Red Cell Abnormalities‡	Platelets	Symptoms§
			Polymorphonuclear neutrophils	Lymphocytes	Large Mononuclears	Eosinophils	Basophils					
Group 1a	21	127	40.0	30.5	26.5	2.5	0.5	25	4.4	++	+	+
Group 1b	4	10.2	41.0	35.5	22.0	1.0	0.5	8	4.9	0	+	0
Group 2	23	10.3	62.5	27.0	8.0	1.5	1.0	5	5.0	0	N+	0
Group 3	20	10.2	60.0	29.0	9.0	1.5	0.5	5	4.3	+	N	0

* The figures and data given represent an average obtained at the time the large mononuclear cells were highest or near their height in each instance. Group 1—Composed of individuals whose large mononuclears were over 12 per cent, a, with symptoms due to tetrachlorethane, b, without symptoms due to tetrachlorethane. Group 2—Composed of men, and Group 3 of women, whose large mononuclears were less than 12 per cent.

† Representative average counts, entire group not always included.

‡ +, indicative of the very slightest definite red cell abnormality, such as due to a very mild degree of secondary anemia from any cause, and as often found in the women employees, ++, indicates a rather greater degree of red cell abnormality, such as referred to in the legend of Table 1.

§ + means the presence of symptoms due to tetrachlorethane, and 0 that there were no such symptoms.

weeks, at which time preliminary symptoms of poisoning occur. Four subjects developed over 40 per cent of large mononuclears, the highest count recorded being 56 per cent. The nine more severely poisoned subjects had usually, but not always, a slightly higher percentage of large mononuclears than the twelve very mildly poisoned workmen. In a typical case, though the symptoms of poisoning may progress over a period of a few days, there occurs no further increase of the large mononuclear cells. These cells gradually fell from the vicinity of 40 per cent to a level of about 12 per cent in from three to six weeks, while if they rose to only about 25 per cent, they fell in a shorter period of time. Their diminution is nearly always to be regarded as

a desirable sign, but, perhaps, this may not always be the case, as will be referred to later. Sixteen of the members of Group I were of the typical sort. However, our data shows that there may be marked variation from the typical case which would appear to be somewhat dependent on the persons resistance to the poison, and to the amount of exposure to it. The worker may show the same progression of blood changes and symptoms, but develop the blood changes over a period of less than two weeks instead of over from four to eight weeks. This is unusual and occurred in only one instance. Again, an individual may have over a period of months a relatively high level of his large mononuclears (from 10 to 20 per cent) and then suddenly symptoms will develop coincident with a rapid rise of the large mononuclears. This may occur, as it did in one case, without realizing that any increased exposure to the poison had occurred, or, as occurred in another case, a rapid rise from 10 to 40 per cent occurred, coincident with the acute development of symptoms due to increased exposure to the poison. In such an instance, the symptoms may develop too rapidly to be foretold by the usual routine blood examination. However, such cases show the characteristic blood picture, which returns to normal in the usual manner.

From observations of two persons developing an acute respiratory infection it was apparent that an intercurrent infection might lower the resistance to the poison. This was evidenced by the fact that their mononuclears rose in about twenty-four hours from about 10 per cent to approximately 40 per cent. Symptoms believed to be dependent on the poison then developed which were sufficiently ill defined to be plausibly explained as due to the infection, as similarly the blood picture might be interpreted. Coincident with removal from the poison, and with subsidence of the infection, the mononuclear count fell somewhat more rapidly than was usual in the typical case.

In spite of these variations from the typical case, it is to be borne in mind that, as a rule, the insidious progressive onset of poisoning is the commonest form, the one to guard against and the one in which symptoms can be foretold from the blood changes.

The fact that the occurrence of clinical poisoning can often be foretold by the blood from something more than an increased percentage of large mononuclear cells, must be borne in mind. It may be foretold by progressive increases of these cells, together with changes in their character and other blood features yet to be commented on.

An increase of large mononuclears, on the average as great as occurred in the presence of symptoms, occurred in a few cases some months before symptoms of poisoning developed, though before and after the appearance of such symptoms there was a progressive increase

of the cells as well as changes in their character. This indicates that any employee carrying an abnormally high mononuclear count is to be regarded as potentially poisoned and may at any time develop clinical symptoms.

Four men, as previously noted, those of Group I b, showed an increased percentage of large mononuclears without developing symptoms of poisoning. Two of these were men about 45 years of age, who had been exposed to the poison upward of a year, and who showed an increased percentage of their large mononuclear cells for three months. The two others were boys about 17 years of age, who had been exposed to tetrachlorethane for from twenty-four to forty-eight hours. The increase of the large mononuclears in the older men may be explained on the ground of a constant chronic irritation by tetrachlorethane—a calling out of the first line of defense. This is further suggested by the fact that when these men changed their work to a place of less exposure, their large mononuclears became markedly reduced over a period of four months. The case of the boys, whose large mononuclear count fell nearly as rapidly as it rose, although they continued at work, we may easily assume as being due to a reaction to the substance. It would seem that as their bodies found they could functionate without the need of so sharp a reaction to tetrachlorethane, the count fell.

A high mononuclear count appears to be the earliest sign of a reaction to tetrachlorethane. The mere fact that there is an increase of the large mononuclears is not sufficient evidence to advise that a man be employed in different work. A high count alone, which does not become progressively higher has not nearly the same significance as a count rising slowly, particularly over a relatively short period of time, except apparently, when persons are first exposed to the poison.

Besides the fact that a progressive increase of large mononuclears is more significant of a deleterious effect of tetrachlorethane than a stationary level of these cells, there is another alteration in the large mononuclears that is significant in indicating the clinical prognosis. This is referable to their detailed character. It was found that there was a rather distinct relationship between the youth of the large mononuclears and the degree of clinical poisoning. With an increase of large mononuclears, the greater the number of young large mononuclear cells, the sooner there developed clinical evidence of poisoning. While if symptoms were present and increased, there occurred an increase in the number of young, large mononuclears.

We consider that the younger large mononuclear cells are differentiated from the older in the following ways. The nucleus of younger cells is oval or round and usually less homogeneous in contrast to the indented or lobed rather dense nucleus of the adult cell. The more

youthful cells are usually of a different size than the more mature ones, they are sometimes smaller, with the cytoplasm relatively less in amount in proportion to the nucleus than in the adult cell, but on the contrary, are usually larger with a relatively large amount of cytoplasm. It is characteristic for the cytoplasm of the younger forms to be more basophilic and contain fewer azurophil granules. A certain amount of difficulty was experienced in making accurate differential counts in these cases, dependent on the great variation in the character of these cells, as indicated above. At times, differentiation from derivatives of the lymphoid system is perhaps impossible. The use of Goodpasture's modification of Graham's oxydase stain, which stains the granules of cells of myelogenous origin, and not those of lymphocytes, was found of assistance as a supplement to the Wright's stain.

An increased fragility to mechanical injury (as from pressure of a cover glass) of white cells may be regarded as evidence of youth. Cells that have become broken cannot be classified in a differential count, but when one can trace a series of cells from those that are intact to those that are broken, one may conclude that the majority of such broken cells are of the type to which they can be traced. In our preparations the majority of the broken or fragile white cells could be interpreted best as large mononuclears. The frequency of these disintegrated cells has been indicated in the tables. They were found quite consistently in increasingly greater numbers as the clinical symptoms appeared or progressed, and were associated with increases of large mononuclear cells showing the other signs of youth. The increases of these fragile forms occurred with too great regularity to be considered purely due to careless technic, though, of course, a few such cells, some of which are probably adult cells, will occur in practically any blood smear.

The numbers of these distorted cells, together with the number of other youthful large mononuclears is to be regarded as a very definite factor in forming a prognosis. The more there were of such cells, indicating increased activity of the tissue from which they are formed, the sooner definite symptoms occurred or more serious symptoms developed. It was common to find from three to seven days before symptoms developed, from ten to twenty broken cells to one hundred formed white cells. With the presence of definite symptoms they increased, on the average to about forty per 100 formed white cells, while in some of the more marked and more acute cases of poisoning they reached to over 100 per 100 formed white cells. No case of poisoning showed less than fifteen fragile cells to 100 formed white cells. Likewise, the formed large mononuclears in the poisoned individual had very varying characters, some were very young and others were mature, with all variations between these extremes. In contrast to this picture, those four persons who did not develop symp-

toms, and those poisoned persons who had high large mononuclear counts, some weeks or even months before poisoning occurred, showed at such times usually only about ten fragile to 100 formed white cells. There was also little variation in the character of their mononuclears, which were particularly of the more adult forms. In other words, when symptoms of poisoning occurred there was much greater activity of formation of the large mononuclears than when symptoms did not occur, even though they might be increased in numbers, indicating a reaction to tetrachlorethane.

It is evident that increases of the large mononuclear cells indicate a reaction to tetrachlorethane and appear to be an early sign of poisoning which may occur before clinical symptoms are apparent, while the youth of these cells, as told by their histologic appearance, is a sign that indicates somewhat the degree of poisoning. These signs are of considerable importance in deciding whether a man should be laid off from work exposing him to tetrachlorethane, and, if so, for how long. Before formulating rules as to how blood examinations may be used to prevent poisoning and for its diagnosis, it is desirable to describe the other less important alterations of the blood that were observed in these workers. These other blood changes are referable to the total leukocyte count, further alteration in the differential leukocyte count, the red cells and platelets.

Accompanying the increase of large mononuclears, there occurred a slight, absolute increase of the leukocytes, never above 22,000 per cm and usually about 12,000 per cm. The higher counts were apt to be found coincident with the greater increases of the large mononuclears and when they showed the greater histologic changes from normal. Among twenty-three individuals exposed to tetrachlorethane, but presenting no signs or symptoms of poisoning, and who had no other blood changes, the white count averaged 10,300. These observations suggest that anyone who comes in contact with tetrachlorethane is very apt to have a slightly elevated leukocyte count, and thus this chemical tends to cause an increase of the total number of white cells.

Alteration in the differential count, other than alteration in the large mononuclears, is of relatively slight importance. Often there was found an initial relative decrease of the polymorphonuclears, without particular fluctuation of the lymphocytes, coincident with the increase of large mononuclears. With a decrease in the percentage of large mononuclears, the lymphocytes frequently rose more rapidly than did the polymorphonuclears, often reaching a percentage higher than that preceding the initial changes due to tetrachlorethane. It is our impression that should a person become distinctly severely poisoned (one example of which we have seen, but who was not in the group of workers we have especially studied) that the large mononuclear

cells may fall, the defensive reaction having been overcome, and one may then find a definite lymphocytosis. Thus, if the clinical symptoms of poisoning were progressive, and the large mononuclear count was falling, while the lymphocytes were rising, it should not be necessarily interpreted that the individual was getting better, but that he was getting worse. In morphology, the lymphocytes seemed to be for the most part normal. In some cases, particularly with an increase in their numbers, there was a somewhat increased percentage of large lymphocytes, some of which were atypical.

Besides alterations in the percentage of polymorphonuclear neutrophils referred to above, the only other abnormality they presented was that at times young forms were found in the peripheral blood. Young forms in rather small numbers were especially seen when there was evidence of considerable heightened activity of the formation of large mononuclears.

No definite variation from normal was found in the polymorphonuclear eosinophils or basophils.

The abnormalities of the red cells that were observed were distinctly of secondary interest and importance as compared with the abnormalities in the white cells. The average red count in eleven men (twenty-five counts (with symptoms of poisoning, was 4,400,000 (varying from 3,900,000 to 5,200,000), while in ten men (ten counts) who had no increase of their large mononuclears, and who had no symptoms, the average red count was 4,900,000 (varying from 4,700,000 to 5,200,000). The average count among nine women (nine counts) who had no symptoms of poisoning, and who for the most part were typically pale factory girls, was 4,300,000 (varying from 4,100,000 to 4,600,000). No accurate hemoglobin determinations were made. By the Tallquist scale, it was apparent that there was a slight reduction of hemoglobin that roughly paralleled the red counts. Abnormalities of the histology of the red cells in the poisoned cases were more evident than the variation from normal of the red count and hemoglobin. The amount of achromia, variation in size and shape and polychromatophilia, that was observed in the average case has been indicated in the tables. There was no evidence of abnormal fragmentation of the red cells, such as occurs in trinitrotoluene poisoning, to suggest that any important degree of blood destruction occurred in tetrachlorethane poisoning. In no case, were the abnormalities of the red cells present to more than a slight, yet definite, degree. The abnormalities were distinctly more apparent in the men developing poisoning, whose red counts, in turn, were lower than those of the men who did not develop poisoning, yet higher than in the women who were not poisoned.

All the instances of this simple anemia, unexplained by some other cause than due to tetrachlorethane, and as particularly told by the

histology of the red cells, developed in cases that showed increases of large mononuclears or clinical symptoms of poisoning. However, anemia did not develop in every case of poisoning. The red cell changes were somewhat more marked in the more chronic and more severely poisoned cases. From our data it appears that the anemia is slowly progressive, especially when symptoms persist or with recurrence of poisoning, but it does not become serious. In any given case, the amount of red cell abnormality alone would be of very slight importance in judging the state of the worker. However, the presence of red cell abnormality is to be regarded as an undesirable finding, and particularly when present with the more important white cell abnormalities, it is to be considered an additional sign to indicate that the worker should be removed from the poison. It is also probably wise to consider that the poisoned individual with anemia should remain longer from exposure to tetrachlorethane than one without anemia, and that persons with anemia should not be exposed to this substance.

The blood platelets as estimated from fixed smears were frequently found slightly increased above normal. As might be expected, their increase was usually more apparent in those cases showing distinct abnormalities of the large mononuclears and changes in the red cells, than in those with lesser changes in these elements. Abnormally large platelets were rather frequently found in cases showing other abnormal blood histology.

From this study of the blood of tetrachlorethane workers the hopes that blood examination might be of value in regulating the employment of those exposed to this poison, have been rather definitely fulfilled.

On the basis of the correlated study of the clinical picture and the blood, the following recommendations regarding blood examination are made for the prevention and diagnosis of poisoning by tetrachlorethane. These recommendations apply when the substance is used under such satisfactory conditions as were observed during our investigation.

A routine blood examination should be made before a person is accepted for work. This will particularly establish his normal level of large mononuclear cells and will determine the presence or absence of any anemia. As anemia probably favors the development of poisoning, if there is any definite degree of it the persons had best be rejected.

A critical examination of a stained blood smear should be made on each new employee at the end of the first and second week, and then once every three or four weeks during the first six months of his exposure to tetrachlorethane. After the first six months, less frequent examination of the blood is necessary. It appears that a given individual is much more likely to develop evidence of poisoning during

the first six months of his exposure. This is substantiated by the fact that in the subjects examined, seventeen cases of poisoning occurred among the men employed for less than six months, while only four cases occurred in a group of essentially the same number who had been employed for more than six months. A new employee may have an initial reaction to the poison. In the event of his being distinctly susceptible, the blood picture may progress from that point. In the event of his being less susceptible, his initial mononuclear rise subsides and probably some tolerance is established.

If the routine blood examination reveals the large mononuclear cells above 12 per cent, more frequent examination is indicated. The frequency of such examinations will depend on the relative rate of increase of the large mononuclear cells together with their numbers showing signs of youth, as well as the presence of even the most trifling clinical symptoms.

Any individual, with a percentage of large mononuclear cells of more than twelve must be considered, for the purposes of diagnosis and prognosis, potentially as a poison case, because an increase of the large mononuclear cells seems to be the first sign of a reaction to tetrachlorethane. However, this arbitrary figure of 12 per cent cannot be utilized alone in regulating his employment, except ideally and impracticably. From the point of view of the employer, such regulation would result in serious economic loss, and from the outlook of the employee injustice would be done. Also, it is evident that no recognizable harm comes to the employee who is allowed to reach the point of developing the very earliest clinical symptoms of poisoning.

We have already noted that the large mononuclears in the twelve mildest cases were often as high as in the nine more severe cases, who were removed from work. However, in these more severe cases the presence of larger numbers of younger mononuclear cells and broken cells was very much more constant than in the twelve milder cases. The determining factor in the removal of the nine men was not only that the blood changes were more marked but that the clinical symptoms were more marked. In both instances, it was on account of the blood examination that these persons came under critical observation, some, however, were allowed to develop a greater degree of poisoning than we now feel is desirable to permit. Of the nine severer cases, all but the two acute ones could have been removed from work sooner than they were, and should have been from the evidence of poisoning obtained from the blood as well as by symptoms.

The regulation of employment, therefore, from the point of view of the blood findings has to be based particularly both on the relative increase of the large mononuclear cells over a definite period of time and on the number of the young and fragile forms.

It may be stated that a progressive increase of large mononuclear cells alone does not call for removal of an individual from work, but calls for his close observation. If the slightest clinical symptoms develop, it is desirable to remove him from exposure. It is permissible, however, to allow him to continue at work for a few days unless his symptoms increase in the least, or his blood picture becomes more abnormal, as particularly evidenced by an increase of the formed and broken young large mononuclears. Any person having symptoms, even if very slight, for more than a few days, with abnormalities of the blood, should be excused from work. An increase of large mononuclear cells, from 12 to 20 per cent, or from 20 to 40 per cent, for a period of two or three weeks, is to be considered as of much more significance than a similar increase for a period of from two to three months.

In the same way that the blood serves as an important guide to indicate when to take an employee off work, so it serves in regulating his return. No person should return as long as any clinical symptoms persist, with the possible exception of a very slight and subsiding jaundice. If the subjective symptoms have subsided and the large mononuclear cells have been reduced by at least 50 per cent from their highest point, then, even, perhaps, in the presence of slight jaundice, if none of the younger forms occur in the blood, a man may be allowed to return to work, provided he is kept under the strictest observation. The absolute percentage value of the large mononuclear cells is of less importance, that is, it does not appear to be absolutely necessary for the large mononuclear cells to return to 12 per cent or less before allowing a person to resume work. The presence of definite anemia is a contraindication to returning to work, and is usually sufficient evidence to advise a change of occupation.

The period of time that the poisoned subjects were removed from exposure varied from a few days to six or even eight weeks. In general, the sooner a man was removed from work after definite blood changes and slight symptoms occurred, the shorter was the time lost.

The reason that these persons should be watched particularly carefully on returning to work after poisoning, is because there seems to be a tendency for those who have become poisoned once to become poisoned again and usually relatively more rapidly than before. If an individual becomes poisoned twice within a few weeks, or several times some months apart, it is wise that he should change his employment. It was found that with the recurrence of poisoning the blood changes were usually more severe and more rapid.¹² As stated, it may be desirable to supplement the routine blood examination with others,

¹² Instances of recurrent poisoning are not included in the previously referred to twenty-one cases of poisoning.

according to the exigencies of the case. These more frequent tests may be made purely because the blood has been abnormal, or because some symptoms have occurred. It is to be clearly understood that every employee developing any symptoms, no matter how trivial, which could in any way be interpreted as due to tetrachlorethane, should have a critical blood examination. Under such conditions the blood will materially aid in the differential diagnosis of the cause of the symptoms. If the typical blood changes described as due to tetrachlorethane are present, the symptoms had best be credited to the poison, while if no increase of large mononuclears occurs, the symptoms can be attributed to some other cause. In this connection it is desirable to note that occasionally certain acute infections and other conditions may cause a considerable increase of the large mononuclears. However, confusion in the diagnosis between tetrachlorethane poisoning and conditions that occasionally give a blood picture simulating that due to tetrachlorethane, must be rare.

We feel that blood examination utilized in the manner outlined above is the most important single criterion we have for indicating the onset of poisoning by tetrachlorethane or its initial symptoms. It is concrete and the important changes that take place are constant. This is in contrast to the very earliest symptoms of poisoning, which are vague and indefinite. Such symptoms, however, are shown to be quite definitely due to poisoning when present with the blood changes described. During our study we have been able to prophesy frequently from the blood some days before clinical symptoms occurred that such symptoms would appear. The blood changes were found to be invariably the first symptoms or signs to appear and other than jaundice, were the last to disappear. However, blood examination is not infallible in detecting poisoning before clinical symptoms develop, because occasionally, from undue exposure, symptoms may develop so rapidly that they could not be anticipated from routine blood examinations.

The acute development of even slight symptoms may be due to a relatively sudden greater exposure to the poison or to the action of some factor not clearly understood, as a lowered resistance from infection. However, such cases will show the typical blood changes so that diagnostically the blood is always helpful.

One should most emphatically not rely on blood examinations alone to keep the health of these employees at its best. It aids to detect the early, often apparently insignificant, symptoms due to poisoning, and serves as an absolute check on them. Blood examination should be utilized as a valuable method to aid in the prevention of serious poisoning, and in the diagnosis and prognosis of poisoning. However, proper clinical supervision of those exposed to tetrachlorethane, as outlined by Dr. Parmenter, is of the utmost importance. If blood

examination is used intelligently, it can aid to reduce materially the time lost from work, for early removal from exposure may reduce the time out to less than a week, while cases that go on to the development of jaundice, usually require at least a month

It is only by careful observation, at least every few days, together with a clear conception of the initial symptomatology of the poisoning, and with utilization and proper interpretation of blood examinations, that poisoning can be kept at a minimum. Under such circumstances, carried out by Drs. Parmenter and Smith, the number of working days lost due to poisoning in this artificial silk plant, has been reduced for the five months preceeding March 1, 1921, by about 40 per cent below what the number was for the five months beginning Jan. 1, 1920, when the original investigation reported here was conducted

SUMMARY

1 A study of the blood of sixty-eight persons exposed to a greater or lesser degree to tetrachlorethane, indicates that blood examination is of value in the prevention of tetrachlorethane poisoning and in the diagnosis and prognosis of poisoning by this substance

2 All persons presenting clinical symptoms of tetrachlorethane poisoning (twenty-one in number) showed characteristic blood abnormalities. The blood changes usually can be observed before clinical symptoms develop

3 The blood abnormalities include (a) a progressive increase of large mononuclear cells, often reaching 40 per cent. This is the most important change. (b) The appearance of many immature large mononuclears. (c) A slight elevation in the white count. (d) A progressive but slight anemia. (e) A slight increase in the number of platelets

4 A percentage of large mononuclear white cells above 12 is the first sign of a reaction to tetrachlorethane, and is a signal for close observation of that person. All persons with such a picture do not necessarily develop clinical symptoms of poisoning

5 The presence of a considerable number of young large mononuclear cells, some formed and many broken, is to be considered as indicating a severer condition than when the same number of more mature large mononuclears are present

6 A consideration has been given to the utilization of blood examinations in connection with careful clinical observations in the regulation of employment of those exposed to tetrachlorethane

THE EFFECT OF THE EXTRACT OF THE POSTERIOR LOBE OF THE PITUITARY ON BASAL METABOLISM IN NORMAL INDIVIDUALS AND IN THOSE WITH ENDOCRINE DISTURBANCES*

C A MCKINLAY, M D

MINNAPOLIS

That there is an interrelation of the glands of internal secretion is well known Rowntree¹ states that where life is, metabolism is, and regulating it are the endocrine glands Kestner² states that all endocrine glands are closely related and that the most intimate relation exists between the thyroid and hypophysis Engelbach³ mentions as a fact that the hypothyroid state is frequently associated with hypopituitarism. Peritz⁴ thinks that the intensive growth in length of bones which is sometimes found in exophthalmic goiter may be ascribed to a functional disturbance of the hypophysis Holmgreen⁵ mentions that exophthalmic goiter patients in youth may show accelerated growth in height Friedman⁶ believes that there may be a mild overactivity of the hypophysis in exophthalmic goiter and a mild underactivity in myxedema

Numerous growth and feeding experiments give evidence of the relationship between the endocrine glands Rogowitsch⁷ concluded that the changes in the hypertrophied hypophysis which follow thyroidectomy in mammals is indicative of a gland of low function Fry⁸ points out that the histology of the hypophysis in exophthalmic goiter corresponds to a gland of high activity, in myxedema to one of low activity Larson⁹ has fed the anterior lobe of the hypophysis to thyroidectomized

* From the Department of Medicine, the Medical School, University of Minnesota

1 Snell, A M, Ford, F, and Rowntree, L G Studies in Basal Metabolism, J A M A **75** 515 (Aug 21) 1920

2 Kestner, O Innere Sekretion Ztschr f artztl Fortbild **17** 573, 1920

3 Engelbach, Wm Classification of disorders of the Hypophysis, J Endocrinology, **4** 347, 1920

4 Peritz, G Akromegalie and Gigantismus, Kraus und Brugreh **33** 633

5 Holmgreen Quoted by Falta Myers, p 83

6 Friedman, G A Suggestions Regarding the Rôle of the Hypophysis in Graves' Disease and in Myxedema, New York M J **113** 370, 1921

7 Rogowitsch, U Die Veränderungen der Hypophyse nach Entfernung des Schilruse, Beitr z path Anat **4** 453, 1889

8 Fry, H J B The Pituitary Gland in Diabetes Mellitus and Disorders of the Glands of Internal Secretion, Quart J M **8** 277, 1914

9 Larson, J A On the Functional Correlation of the Hypophysis and the Thyroid, Am J Physiol **49** 55, 1919

rats and finds that it has a beneficial effect on their growth Kojima¹⁰ found in rats after thyroidectomy hypophyseal changes indicative of a gland of low activity Hart¹¹ noted atrophy of the thyroid in tadpoles fed with thymus, he states that monoglandular disturbance is often followed by changes in the other organs of the endocrine system and that what would appear to be monoglandular diseases are often polyglandular

Certain tests to aid in diagnosis have been described which have their rationale in endocrine interrelationship According to Goetsch,¹² who revived the work of Eppinger and Hess,¹³ the hypersensitiveness of the sympathetic nervous system to epinephrin has been made the basis of his epinephrin test Ascoli and Faguoli¹⁴ describe a test consisting of subcutaneous injection of pituitary extract with positive local reaction strengthened in affections of the pituitary body The reaction is often entirely dissociated from that to adrenalin which they also describe Porak¹⁵ found that injection of the extract of the posterior lobe of the hypophysis had much more effect on the blood pressure of myxedematous patients than of normal subjects as shown by sudden and prolonged fall of tension The observation was also made that injection of thyroid extract in normal subjects causes lessened blood pressure, but not in individuals with myxedema Porak thinks that it is possible by these tests to confirm or exclude hypothyroidism in complex syndromes Boothby and Sandiford¹⁶ have shown that epinephrin causes an increased heat production in the body, and conclude that there is no apparent relationship between cases positive to the Goetsch test and hyperthyroid states as measured by their basal metabolic rates Cushing¹⁷ states that in the early stages of acromegaly there is a definite increase in metabolism, whereas in the reverse states the rate averages at least twenty points below normal Engelbach³ seems to conclude that there is usually increased basal metabolism in hyperpituitarism Snell, Ford, and Rowntree¹ state that in a small series of cases no profound or lasting effect on basal metabolism resulted from

10 Kojima, M Studies on Endocrine Glands, Quart J Exper Physiol **51** 319, 1917

11 Hart, C Berlin klin Wchnschr **57** 101, 1920

12 Goetsch, E Newer Methods in the Diagnosis of Thyroid Disorders, New York State J M **18** 259, 1918

13 Eppinger, H, and Hess, L Zur Pathologie des vegetativen Nerven Systems, Ztschr f klin Med **67** 345, 1909

14 Ascoli, M, and Faguoli, A Pituitrin Test, Endocrinology, **4** 33, 1920

15 Porak, R Physiological Action of Different Extracts of Endocrine Glands in Myxedema, Ann de med **6** 469, 1920

16 Boothby, Wm, and Sandiford, Irene Abst Proc Am Physiol Soc, Am J Physiol **51** 200, 1920, in full Ibid **51** 407, 1920

17 Cushing H Disorders of the Pituitary Gland, Retrospective and Prophetic, I A M A **76** 1721 (June 18) 1921

the subcutaneous use of the extract of the posterior lobe of hypophysis. Their investigation did not include its immediate effect when used subcutaneously.

To determine what influence the extract of the posterior lobe of the hypophysis when used subcutaneously has on heat production in normal persons and in those with endocrine disorders, chiefly of the thyroid and pituitary, has been an object in this study. Whether thyroxin,¹⁸ the active principle of the thyroid gland, and pituitary extract have any interrelated effect on basal metabolism has been a point of extreme interest.

Material and Methods Used—The gasometer method of obtaining the basal metabolic rate has been used and air analysis was made with the Haldane apparatus. The usual precautions have been observed to have the patient in the postabsorptive stage, that is, determinations were made in the morning fourteen hours after any food intake. The patients have been subjected to a twenty minute rest period while lying down. After such preparation this routine method has been employed: (1) Collection of air for the initial determination of the basal metabolic rate, (2) followed within ten minutes by a subcutaneous injection of pituitary extract, (3) collection of expired air started twelve minutes after injection. The preparation used has been the extract of the posterior lobe and pars intermedia, approximately 1 cc in amount, except as otherwise indicated in the text. The collection of the expired air has averaged between eight and ten minutes. Especial care has been taken during the experiments to control all extraneous factors that might alter the patient's basal metabolism during the routine procedure. In view of increased restlessness which is sometimes apparent after protracted rest periods, a control series has been included.

This group consists of five normal persons who were subjected to the routine procedure mentioned above, except for the omission of the pituitary extract between the two determinations. In Table 1 are shown the results in which the variation in the basal metabolic rate in each individual has never been more than one per cent in the two determinations. Such a small variation appears to be a negligible factor.

In the cases given thyroxin administration was intravenously in 10 mg dosage. One week later, approximately at the height of the accelerating effect of thyroxin on basal metabolism, pituitary extract was administered according to the routine procedure before described, with the purpose of observing the combined physiologic effects of the two products.

18 Kendall, E. C. The Isolation in Crystalline Form of the Compound Containing Iodin Which Occurs in the Thyroid and its Chemical Nature and Physiological Activity, *Tr. Ass. Am. Phys.* **33** 420, 1915.

The individuals on whom the experiments have been performed have been grouped as follows (1) Normal, (2) hypothyroid, (3) pituitary disorders, and (4) normal and hypothyroid cases given thyroxin. The description of cases is given under each group.

TABLE 1—NORMAL INDIVIDUALS USED AS CONTROLS WITH TWO SUCCESSIVE DETERMINATIONS OF THE BASAL METABOLIC RATE

No	Initial Determination	Second Determination	No	Determination Initial	Determination Second
1	-3	-4	4	+0.5	+1
2	-9	-10	5	+1	+0
3	+1	+0.5			

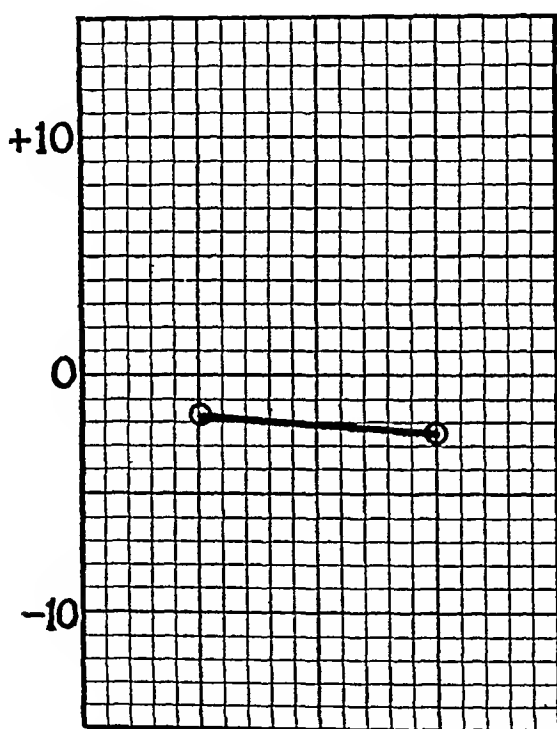


Fig. 1—Control Group. Average of two successive determinations of basal metabolic rate without the use of pituitary extract in five normal individuals.

DISCUSSION

The normal individuals included in Group I, a majority of them students, have shown no evidence of endocrine disturbance, and their basal metabolic rates were all within normal limits. Eleven out of twelve responded with increased heat production following the use of pituitary extract. The percentile increase in basal metabolism varied from 2 to 16 and averaged 5. No pronounced effect was noted on the rate of respiration. The total ventilation was generally increased. Blood pressure determinations revealed slight systolic elevation in some

subjects, entirely absent in others. Quite marked pallor has been noted in many of the patients within twenty minutes following the use of pituitary extract. Subjectively abdominal cramps have been commonly noted.

TABLE 2—NORMAL INDIVIDUALS GIVEN PITUITARY EXTRACT

No	Initial Determination	Determination After Injection	Determination 20 Minutes Later
1	-2	+2	
2	+10	+17	
3	+3	+7	
4	+3	+7	
5	+1	+3	
6	-12	-4	-9
7	-5	0	
8	+7	+13	
9	+2	+2	
10	-7	+9	
11	+1	+5	
12	-10	0	

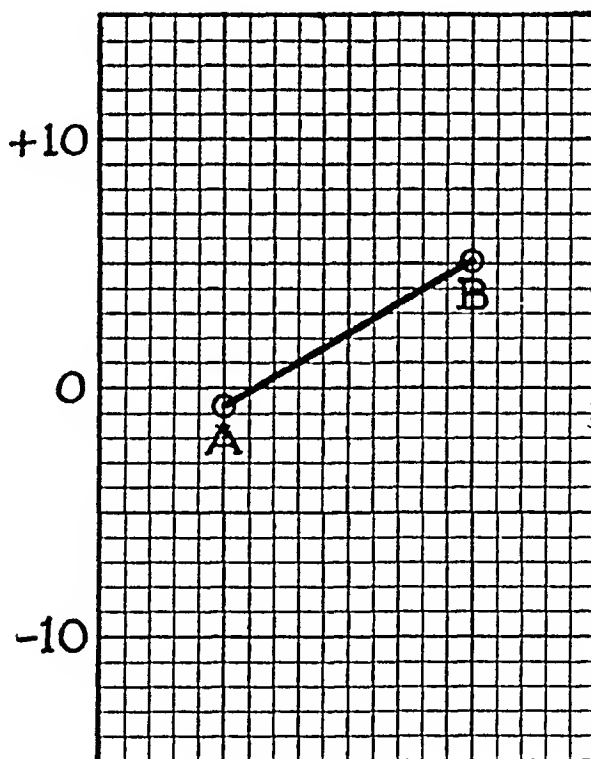


Fig 2—Effect of pituitary extract on twelve normal individuals, average basal metabolic rate, A=before, B=after use of pituitary extract

Group II consists of four cases of hypothyroidism. Their response to pituitary extract is intensely interesting. Three of these are cases of myxedema whose manifestations were mild, judged by clinical signs and basal metabolism. Heat production in these cases was diminished

rather than increased. Another case of hypothyroidism developing after lobectomy showed similar response following the use of pituitary extract. In this group the subjective symptoms were less marked than in the normal individuals, no elevation of blood pressure being observed, which is in keeping with the findings of Porak¹⁵. In marked contrast to its accelerating influence on basal metabolism in normal individuals pituitary extract was entirely inert in a group of four subjects with hypothyroidism. It seems, then, that pituitary extract is not effective in accelerating heat production in cases with inactivity of the thyroid. This observation may be taken as evidence that there is a synergy between the thyroid and the hypophysis, if we may judge from the influence pituitary extract has on normal thyroid activity in accelerating basal metabolism. The explanation of the cause of such an interrelation is not clear at the present time and therefore demands further investigation.

TABLE 3—HYPOTHYROID PATIENTS GIVEN PITUITARY EXTRACT

No	Initial Determination	Determination after Pituitary Extract	No	Initial Determination	Determination after Pituitary Extract
1	—15	—17	3	—19	—22
2	—11	—15	4	—14	—16

Group III includes three cases in which disturbed function of the hypophysis was evidenced by the development of obesity, increased sugar tolerance, subnormal temperature, and dysmenorrhea in one of them. Though in these cases basal metabolism was diminished, the thyroid was apparently normal judged by absence of signs of myxedema. In these cases, as in normal persons, pituitary extract accelerated heat production. In such relationship holds true in a larger series of cases, it is suggested that the determination of the effect of pituitary extract on basal metabolism may be used as a test to estimate in pluriglandular cases the extent, if any, of thyroid involvement. In the small series of cases observed, it seems to hold true that cases with low heat production, associated with frank thyroid disturbance, do not respond to pituitary extract while those associated rather with hypophyseal disturbance respond as do normal persons with increased basal metabolism.

Group IV consists of cases in which thyroxin was administered intravenously, followed in one week by pituitary extract subcutaneously. The normal individuals included in subdivision "A" all responded with an increase in basal metabolism which amounted to 8 per cent. This should be compared to 6 per cent in a similar group without thyroxin. Apparently, then, in four normal individuals thyroxin has increased the accelerating influence of pituitary extract on basal metabolism. On

the other hand, two patients with myxedema, included in subdivision "B," who had likewise received thyroxin with subsequent increase in heat production, showed no response to pituitary extract. In two cases with subnormal thyroid activity pituitary extract, then, has been entirely ineffective in accelerating heat production. Such ineffectiveness persisted when the thyroid was temporarily stimulated to activity by thyroxin.

TABLE 4—PATIENTS WITH PITUITARY DISTURBANCE GIVEN PITUITARY EXTRACT

No	Initial Determination	Determination after Pituitary Extract	No	Initial Determination	Determination after Pituitary Extract
1	-15	-1	2	-12	-9
	-23	-15	3	-14	+1

Whether any conclusion can be drawn from the findings that a group of normal subjects given thyroxin responded with a greater increase in basal metabolism than those without it, is doubtful. Taken, however, with other observations it suggests that there is a synergic action between pituitary extract and thyroxin and that such action depends upon a normally functioning thyroid. The experiment may, then, be interpreted to give evidence that heat production of the body is due to a balance between endocrine glands, more specifically between the thyroid and hypophysis.

TABLE 5—PATIENTS GIVEN THYROXIN FOLLOWED ONE WEEK LATER BY PITUITARY EXTRACT

Subdivision A—Normal individuals

No	Initial Determination	Determination after Pituitary Extract	No	Initial Determination	Determination after Pituitary Extract
1	+16	+18	3	+21	+29
2	+12	+20	4	+11	+24

Subdivision B—Hypothyroid Cases

No	Initial Determination	Determination after Pituitary Extract	No	Initial Determination	Determination after Pituitary Extract
1	+16	+13	2	+9	+9

COMMENT

While the number of cases observed has been limited, it has been shown that in normal persons there is quite constantly increased heat production following the subcutaneous use of pituitary extract within certain time limits. In the group with hypothyroidism the evidence is strongly suggestive that there is lacking this positive response to

pituitary extract and the findings stimulate one to further observations on a larger series of cases. It is suggested that the response following pituitary extract may be used as a test to aid in the determination of the extent of thyroid involvement in pluriglandular disturbances. The evidence at hand is interpreted as indicating that there is a synergic action between thyroxin and pituitrin, the recognition of which may be of therapeutic as well as of diagnostic value.

CONCLUSIONS

1 Normal persons responded quite constantly with increased basal metabolism following the subcutaneous injection of pituitary extract.

2 In a small series of cases with hypothyroidism the basal metabolism was diminished rather than increased, which suggests that pituitary extract is effective in accelerating heat production only in the presence of a normally functioning thyroid gland.

3 In four cases with subnormal basal metabolism in which clinical evidence of myxedema was lacking and preponderance of influence of endocrine glands other than thyroid was suggested, the positive response to pituitary extract was present.

4 The increased acceleration of basal metabolism in a group of normal individuals following the subcutaneous injection of pituitary extract one week after an injection of thyroxin is interpreted as suggesting a synergic action between thyroxin and pituitary extract.

CAPILLARY POISONS AND ACIDOSIS^{*}

GEORGE B WALLACE, M D, AND EMIL J PELLINI, M D
NEW YORK

INTRODUCTION

In his admirable review of the functional activity of the capillaries and venules, Hooker¹ brings out the facts that these structures change in caliber independently of the larger vessels, that they function actively and are responsive to both chemical and nervous influences. He pictures a nervous control, maintaining a tone of the capillary beds over the body as a whole, and a chemical regulation, usually local in character and effective according to the passing need of particular tissues.

As Hooker points out the story of capillary function is far from complete. What facts are known have to do with contraction and dilatation, and the question of capillary permeability is practically untouched. In an ultimate analysis, the function of the capillaries is to allow of the passage of oxygen and nutriment from the blood to tissues and the removal of waste products from tissues to the blood. A physiologic permeability is essential, therefore, for the nutrition and activity of tissues, and the complete mechanism of dilatation, contraction and pressure changes is for the purpose of obtaining and regulating this permeability. The facts bearing on this phase of capillary function are extremely meager.

A knowledge of physiologic function is often added to materially by a study of pathologic changes and their effects. Of special importance in connection with capillary function is the study of the early stages of acute inflammation. It may be assumed that inflammation occurs only when some injurious agent introduced from without or formed from damaged cells comes in contact with an area of tissue. As MacCallum² points out, the first evidence of inflammation consists in a widening of the visible capillaries and the opening of new, hitherto unseen capillary areas. At this time there is an increase in the rate of blood flow. These changes are the ones usually described as following the application of any chemical irritant. Very shortly, however, the rate of flow becomes slower, with the red cells occupying the middle of the capillary channel, and plasma and leukocytes occupying the marginal borders. Obviously this change is due to some alteration

^{*} From the University and Bellevue Hospital Medical College.

¹ Hooker. *Physiol Rev* **1** 112, 1921.

² MacCallum. *Textbook of Pathology*, Ed 2, p 136.

in the capillary structure Further, and probably from the beginning, a change in permeability occurs, which is demonstrable through the passage of fluid and leukocytes into the tissue

The nature of the capillary change in inflammation is not clear Klemensiewicz³ believes that there is a paralysis of the capillary walls and supports this by showing that they fail to respond to nervous or direct stimulation A simple paralysis would fail to explain the whole process, and Samuel,⁴ who holds that the tissue lesions are always secondary to the vascular ones, concludes that there is primarily some unexplained but specific effect on the capillary walls In accepting this rather vague hypothesis it is evident that the specific effect may be induced by a vast number of chemical substances whose only common character is that they are irritants This group produces its effect when applied locally, the effect comes on promptly, and the extent of injury is proportional to the concentration of the irritant and the length of time it remains in contact with the tissue acted on It must be admitted that although the capillary walls may be especially sensitive to the irritant, other tissues may similarly be damaged by direct action But the fact seems clear that in acute inflammation there is primarily an injury to the capillary endothelium which manifests itself not only in change in caliber but in abnormal permeability as well

There is another group of substances which act similarly on the capillaries when brought to them by the circulation and which, in very minute doses, produce wide spread effects The action on the capillaries appears to be as selective as that of strychnin on the nervous system, or digitalis on the heart These substances are classed pharmacologically as capillary poisons The term has a wide application It includes those substances which cause a widening or paralysis of the capillaries without inducing visible structural change It includes also those which produce changes in caliber, accompanied or followed by structural changes As a representative of the first group, histamin may be mentioned This induces a wide spread but transient capillary dilatation⁵ The second group is made up of substances of such varying chemical nature as uranium, cantharidin, emetin, diphtheria toxin, sepsin and gold While many of these are irritants, this property does not explain the wide spread effects produced by minute dosage, unless one conceives a highly specific and selective irritant action This group in its initial action produces a dilatation of capillaries Very shortly, however, a structural damage may become apparent and constitute the main feature While the action appears at times to be localized,

3 Klemensiewicz *Entzündung*, Jena, 1908

4 Samuel *Virchows Arch f path Anat* **121** 273-396, 1890

5 Dale and Richards *J Physiol* **52** 110, 1918

this, as a matter of fact, is not strictly the case, for they may act on capillaries generally. They tend, however, to have a more intense effect on one organ or tissue than on others, this effect overshadowing other, less intense ones. Thus uranium and cantharidin are often considered purely as kidney poisons and emetin as an intestinal poison. The explanation of the evident selectivity of action lies in the excretion of the poison or other factors which determine its concentration. Thus, if given by injection into the jugular vein, the lung capillaries may be the ones chiefly affected, because the poison comes to them in greatest concentration. Those excreted by the kidney produce their greatest effects here, those excreted by the intestine, on that organ. However, if these localized effects are not severe enough to cause early death, changes elsewhere can usually be detected.

Some of these poisons act much more generally than others and this is especially the case with gold salts, sepsin and diphtheria toxin. If the mesentery of a frog is observed under a low power microscope, in from one half to one minute after the injection of gold-sodium chlorid,⁶ new capillary fields suddenly appear, the rate of flow is rapid and then quickly slows. In mammals the veins become strongly distended and hemorrhages are numerous in the liver, spleen, kidney and intestine. With diphtheria toxin⁷ there is marked injection of the small vessels and wide spread destruction of capillaries with outpouring of blood. Sepsin⁸ also causes wide spread hyperemia and hemorrhages. Similar effects may be obtained with emetin,⁹ but these are confined to the stomach and intestine, as is the case with colchicine. Such extreme effects as these must, of course, abolish entirely the function of the capillaries involved.

If it is true that these poisons, when their action is well developed, produce structural injury and impaired permeability of the capillaries, it follows that definite effects from these changes should be obtainable and measurable. Among these effects would be departures from the normal metabolism of tissues supplied by the damaged capillaries, departures proportional to the change in permeability. If the capillary changes come on gradually, are not too intense in their development and are of sufficient duration, it is reasonable to believe that the resulting inadequate nutrition of tissue would result in connective tissue growth and replacement, and in various types and stages of tissue degeneration. This, of course, is not a new idea. It was expressed in 1872 by Gull and Sutton,¹⁰ who believed that a general morbid state, which they

6 Heubner *Arch f exper Path u Pharmacol* **56** 370, 1907

7 Wallace and Myers *J Pharmacol & Exper Therap* **6** 601, 1915

8 Faust *Arch f exper Path u Pharmacol* **51** 248, 1904

9 Wallace and Pellini *Am J M Sc* **152** 325, 1916

10 Gull and Sutton *Med Chir Tr* **55** 273, 1872

termed arteriocalillary fibrosis, may exist, characterized by hyalin fibroid formation in the walls of the minute arteries and hyalin granular changes in the capillaries. This morbid state is attended with atrophy of adjacent tissues, and the contracted kidney of chronic Bright's disease is only a part of the process. This view has been recently again expressed and enlarged by Moschowitz¹¹

Now, while it is easy to surmise a causal relationship between capillary injury and tissue degeneration, it must be emphasized that this relationship, as far as human pathology is concerned, is as yet a theory and not a fact. In the first place, the existence of poisons which might be expected to cause the capillary damage is hypothetical. In the second place, as Oertel has stated, there is no proof at present that vascular changes are the forerunners of tissue degenerations, and not part of a damaging process which affects capillaries and tissue independently. In order to leave the realm of theory and enter that of fact, it will be necessary, first of all, to produce by means of a known vascular poison, capillary injury which will be followed by tissue degeneration, and to vary the latter according to the extent and duration of the former. Experimental work along this line has so far not been very successful. Most of it has been carried on with the purpose of producing chronic nephritis. Dickson¹² has produced a chronic diffuse nephritis, with severe glomerular lesions, by means of uranium. In advanced cases, along with other changes, there was a thickening of the capillary walls. He believes, however, that the vascular damage is of slight importance. On the other hand Harvey,¹³ using parahydroxy phenylethylamin, induced chronic kidney lesions which he considers to be secondary to vascular changes in the renal artery and its branches.

There is one other aspect of the effects of capillary injury, namely, its relation to high blood pressure. The popular conception at present is that at least one type of high blood pressure, the so-called essential hypertension, has as its basic cause a narrowing or obliteration of capillary fields. As is the case with tissue degeneration, this relationship is a very plausible one, but again, is a theoretical conception. The problem does not appear beyond solution, however, and we are at present engaged on it in the hope of eventually getting some positive results.

EXPERIMENTAL WORK

In the course of our work we have looked for capillary poisons which have a wide spread rather than a localized sphere of action. This separation is not as simple as it appears. It seemed to us,

11 Moschowitz Arch Int Med 26 259 (Aug) 1920

12 Dickson Arch Int Med 9 557 (Oct) 1912

13 Harvey J Path & Bacteriol 16 95, 1911

however, that a poison which has a rather general effect on capillaries should bring about some measurable change in metabolism, for a lessened capillary permeability ought to lead to a decrease in cell nutriment, and, included with this, a decrease in oxygen. This latter state could readily induce a condition of suboxidation. The ordinary metabolism experiment throws no definite light on this question. There is an increase in nitrogen elimination and other changes, which admit of various interpretations. It seemed, however, that one distinctive type of metabolic derangement might occur, namely, acidosis, and accordingly we have carried out a series of experiments using a number of capillary poisons, and determining their effects on the alkali reserve of the blood. Our results have been definite enough to make it seem desirable to report them in some detail.

The experiments were performed on adult dogs in a good state of nutrition, which were picked indiscriminately from the available laboratory animals. The drug used was given by subcutaneous, intramuscular or intravenous injection. No restriction in diet was made, although in the longer experiments the animals refused food as time went on. Blood to be tested was withdrawn from the external jugular vein once a day or oftener. The blood was received directly without exposure to air, into a tube containing potassium oxalate and mineral oil. It was immediately centrifuged and the serum analyzed for alkali reserve by the Van Slyke method¹⁴.

We feel it unnecessary to quote all our experiments and are limiting our protocols in general to one typical experiment for each poison tested.

PROTOCOLS OF EXPERIMENTS

Uranium—Dog, weight, 72 kilos, uranium nitrate, 0.021 gm, injected subcutaneously October 5. Alkali reserve before injection 57.7 volume, per cent carbon dioxide. After injection, October 6, 53.8 per cent, October 7, 36.8 per cent, October 8, 29.5 per cent, October 9, 25.3 per cent, October 11, 31.5 per cent, October 12, 23.3 per cent, October 13, exitus.

The twenty-four hour urine output rose from 330 c c on October 6 to 1,084 c c October 10. It then fell and was 115 c c October 12.

Cantharidin—Dog, weight 12 kg, cantharidin, dissolved in weak sodium hydrate solution, injected intravenously as follows: November 23, 5 mg, November 24, 7.5 mg, November 25, 26, 27, 10 mg. Alkali reserve, before injection 58 per cent, after injection, November 24, 58.2 per cent, November 25, 58.4 per cent, November 26, 10 a m, 40.9 per cent, 4.30 p m, 36.2 per cent, November 27, 42.5 per cent, November 28, 30.9 per cent. Animal killed November 28. There was no anuria during the experiment.

Diphtheria Toxin—Dog, weight, 12.1 kg, diphtheria toxin m 1 d 1/500 c c, (obtained from Dr. W. H. Park), 1.4 cc injected intramuscularly at 4.30 p m November 30. Alkali reserve before injection, 55.8 per cent. After injection, December 1, 9.30 a m, 30.3 per cent, 11.40 a m, 18.1 per cent. Exitus, December 1.

We have used in these experiments three capillary poisons, two of which, uranium¹⁵ and cantharidin,¹⁶ act especially on the kidney, the third, diphtheria toxin,⁷ acting equally strongly on the intestinal and liver capillaries, and, in fact, on capillaries generally. The action of uranium and cantharidin is not limited to the kidney, however.

In order to determine to what extent the renal action was concerned in the acidosis, the following experiments were done. In a control animal a double nephrectomy was performed under ether anesthesia and the blood was analyzed during the four following days. In a second animal, a double nephrectomy was performed, and on the following day uranium was injected. We are indebted to Dr W. H. Barber for the operative procedures. The results were as follows:

Nephrectomy—Dog, weight, 15.8 kg, double nephrectomy November 10. Alkali reserve, before operation, 57.7 per cent, after operation, November 11, 49 per cent, November 12, 60.2 per cent, November 13, 61.1 per cent, November 14, 69 per cent.

Nephrectomy and Uranium—Dog, weight 11.25 kg, double nephrectomy November 16. November 17, 33 mg uranium nitrate by subcutaneous injection. Alkali reserve before nephrectomy, 50 per cent, after nephrectomy, November 17, 52.3 per cent, after uranium, November 18, 44 per cent, November 19, 38.7 per cent, November 20, 10 a. m., 33.1 per cent, 2.30 p. m., 30.7 per cent.

It is obvious from these experiments that removal of both kidneys is without influence on uranium acidosis, and that kidney damage is not an appreciable factor in this acidosis.

Emetin—Dog, weight, 13.1 kg, emetin hydrochlorid, 1 per cent solution, injected subcutaneously, 2.5 c. c. daily, October 12, 13 and 14, twice October 15, 3 c. c. twice October 16. Alkali reserve, before injection, 63.6 per cent, after injection, October 12, 63.3 per cent, October 13, 63.3 per cent, October 14, 57.9 per cent, October 15, 57.9 per cent, October 16, 63.5 per cent, October 17, 57.6 per cent. Exitus, October 18.

In another emetin experiment a dog was given 21 c. c. of a 1 per cent solution during a period of four days. At the end of this time, under morphin and light ether anesthesia, blood was withdrawn from the jugular, portal and an intestinal vein at approximately the same time. The alkali reserve was 54.8 per cent, 54.8 per cent and 53.9 per cent, respectively.

Podophyllotoxin—Dog, weight, 11.8 kg, podophyllotoxin, dissolved in weak alkali, injected intramuscularly as follows: October 28, 20 mg, October 29, 30 mg, October 30, 20 mg, November 2, 20 mg, November 3, 30 mg. Alkali reserve, before injection, 57.8 per cent, after injection, October 28, 58.6 per cent, October 29, 57.5 per cent, October 30, 53.9 per cent, October 31, 58.6 per cent, November 1, 55.7 per cent, November 3, 53.8 per cent, November 4, 53.9 per cent, November 5, 44.5 per cent. Exitus, November 5, shortly after last blood examination.

¹⁵ McNider J. M. Res **31** 79, 1912.

¹⁶ Lyon J. Path & Bacteriol **9** 444, 1904.

Emetin⁹ and podophyllotoxin¹⁷ act selectively on the intestinal capillaries, and in the experiments just noted necropsy showed the presence of the characteristic intestinal lesions. There was also congestion of the kidney, but the renal damage was not severe, as the urine showed only faint traces of albumin and no striking diminution in quantity. In the second emetin experiment we had expected to find the alkali reserve in the blood from the intestinal and portal veins lower than in that from the jugular vein. In this we were disappointed and have to conclude that marked damage to the intestinal capillaries fails to bring about an appreciable acidosis.

Arsenic—Dog, weight, 16.7 kg, arsenious acid given subcutaneously in daily dosage of 30 mg, November 18, 19, 20 and 21, intravenously November 22, 30 mg, November 23, 30 mg, November 24, 50 mg, November 25, 70 mg, November 26, 70 mg, November 28, 80 mg. Alkali reserve, before injection, 54 per cent, after injection, November 19, 54.9 per cent, November 20, 55.9 per cent, November 21, 55.7 per cent, November 22, 55.6 per cent, November 23, 55.6 per cent, November 24, 55.6 per cent, November 25, 57.4 per cent, November 26, 46.2 per cent, November 27, 45.3 per cent. Exitus, November 28.

In this experiment, the arsenic acted chiefly on the intestinal tract. There was also well marked congestion of the liver and kidneys, and a beginning fatty change in the liver¹⁸. The acidosis was not marked, and this we attribute to an absence of wide spread capillary damage. In another arsenic experiment we obtained the following result:

Arsenic—Dog, weight 13.4 kg, 60 mg arsenious acid injected intravenously at 11.15 a. m. Alkali reserve, before injection, 50 per cent, at 2.45 p. m., 30.4 per cent. Exitus, 3.15 p. m.

Hydrazin—Dog, weight, 10.6 kg, hydrazin sulphate injected subcutaneously 100 mg daily, November 8, 9 and 10, 200 mg daily November 11, 12, 13 and 14, 400 mg November 15. Alkali reserve before injection 53.8 per cent, after injection, November 9, 51 per cent, November 10, 53.9 per cent, November 11, 55.7 per cent, November 12, 62.6 per cent, November 13, 67.2 per cent, November 14, 67.1 per cent, November 15, 65.3 per cent. Exitus, November 16.

This substance is not classed as a capillary poison. It has a highly selective action on liver tissues, however, producing marked destruction and fatty infiltration of liver cells¹⁹. In another hydrazin experiment comparable to the second arsenic and the diphtheria experiments in regard to time, the following result was obtained:

Dog, weight, 61.8 kg, 600 mg hydrazin sulphate injected subcutaneously at 3.45 p. m. November 5. Alkali reserve before injection, 52 per cent, after injection, 11 a. m., November 6, 72 per cent. Exitus, 1 p. m., November 6.

With this marked damage to the liver, we find, therefore, not an acidosis, but the reverse.

17 Neuberger Arch f exper Path u Pharmacol 28 32, 1890

18 Boehm Arch f exper path u Pharmacol 2 89, 1874

19 Underhill and Klein J Biol Chem 4 165, 1908

As the above described experiments show that neither kidney, intestine or liver injury is responsible for the acidosis we have obtained, we carried out the following transfusion experiment to determine whether the muscle tissue is concerned in the process

January 25 a dog was given 175 c c diphtheria toxin injected intramuscularly. The alkali reserve at 11 a m January 26 was 35 per cent. At 11 30 a m the femoral artery and vein of this dog were connected with the femoral artery and vein of a normal dog, the latter, therefore, supplying the circulation to the poisoned leg. The necessary operative procedures were carried out under morphin anesthesia, supplemented with a small quantity of cocain. Hirudin was used to prevent clotting of blood. The transfusion was maintained for over an hour. The alkali reserve of the normal dog before the experiment began was 55.8 per cent. The figures obtained after 45 minutes of transfusion were: jugular blood, normal dog, 55.4 per cent; diphtheria toxin dog, 31.5 per cent; femoral blood from poisoned leg, 42.6 per cent.

Using the bloods taken from these two animals just before the transfusion, we have estimated the hemoglobin and blood cells, and made the ordinary chemical analysis

	Normal Dog	Diphtheria Toxin Dog
Red blood cells	6,100,000	6,800,000
Hemoglobin	85%	80%
White blood cells	12,400	26,200
polymorphonuclears	82%	78%
mononuclears	18%	22%
Nonprotein nitrogen	26.3 mg	62.5 mg
Urea	16.1 mg	38.4 mg
Creatinin	1.5 mg	1.7 mg
Sugar	117.6 mg	44.0 mg
Whole blood chlorids	500.0 mg	500.0 mg

A comparison of the red blood cell count and hemoglobin shows no difference in the concentration of the blood in the two animals. The diphtheria toxin animal shows a leukocytosis and in addition the altered blood chemistry which is to be expected, since the toxin acts on the kidney as well as other capillaries.

An experiment of this sort is naturally open to some criticism from the technical factors involved, but the results show that muscle is one of the tissues involved in the production of acidosis.

We have assumed that a suboxidation due to impaired capillary permeability is the cause of the acidosis from capillary poisons. It seemed to us that this assumption would be strengthened by obtaining acidosis through suboxidation induced by other than capillary poisons. The following experiments bear on this point.

Sodium Nitrite—Dog, weight, 11.6 kg, received by subcutaneous injection 0.1 gm sodium nitrite January 10 at hourly intervals from 11 45 a m to 3 45 p m, in all 0.5 gm. Alkali reserve before injections, 51.4 per cent, at 4 p m 58 per cent. The blood spectroscopically showed no methemoglobin. On the following day, the animal received 0.2 gm sodium nitrite, at intervals, from

10 45 a m to 12 45 p m, 0.8 gm in all. Alkali reserve before injection, 56.1 per cent at 1 10 p m 49 per cent, at 1 50 p m, 29 per cent. At 1 p m blood showed methemoglobin.

It appears from this experiment that a lowering of blood pressure which must have been induced by the nitrite on the first day of the experiment was not productive of acidosis. This appeared only on the second day when the formation of methemoglobin occurred. Methemoglobin, of course, means cellular asphyxia.

Potassium Cyanid—Dog, weight, 11.3 kg, received 34 mg potassium cyanid January 12 at 11 a m, 22 mg at 11 30 a m, 50 mg at 12 noon. No characteristic symptoms, except vomiting and a slight ataxia occurred until 12 25 p m, when marked dyspnea and muscle twitchings appeared. Alkali reserve before injections, 53.8 per cent, after injections, 12 noon, 27.4 per cent, 12 25 p m, 12.2 per cent. Exitus during night.

Potassium cyanid has been studied quite extensively from the standpoint of its effect on metabolism²⁰. It acts in some unexplained way on tissues, preventing their using available oxygen. It may be noted in this experiment that the acidosis was very definite before any convulsive movements had set in.

We have one other experiment to report. In most of our experiments, as poisoning progressed, there developed a condition of muscular weakness, prostration, narcosis and lessening of the respiratory function. We have reproduced this condition by administering large quantities of morphin. The results are as follows:

Morphin—Dog, weight 10.3 kg, normal respiration, 20 per minute. December 1, a total of 190 mg morphin sulphate given by subcutaneous injection at half hour intervals from 10 45 a m to 5 10 p m. The respiration fell to 11 per minute, slightly labored, with moderate narcosis. Alkali reserve before morphin, 64 per cent, after morphin, at 4 20 p m, 61.5 per cent. On the following day, morphine was again given, at frequent intervals, from 9 55 a m to 3 p m. In all 2 gm morphin were injected. At 3 30 p m respiration was 10 per minute and shallow, moderate narcosis, animal being unable to stand. At this time the alkali was 64 per cent. Death occurred during the night.

The administration of large amounts of morphin, therefore, with the production of narcosis and respiratory depression, does not cause acidosis. Gauss²¹ has recently shown that an actual increase in alkali reserve may occur.

DISCUSSION

In looking over these experiments we find that those poisons which produce a wide spread capillary damage, namely uranium, cantharidin and diphtheria toxin, cause a definite acidosis. The first two of these

20 Richards and Wallace. J Biol Chem 4 179 1908

21 Gauss. J Pharmacol & Exper Therap 16 475, 1921

act most strongly on the kidney, but the fact that uranium is equally effective in causing acidosis in nephrectomized animals speaks against renal damage being an essential factor

Emetin and podophyllotoxin produce very destructive effects along the intestinal tract, and yet neither causes an appreciable acidosis. Again, hydrazin is a powerful liver poison, but its administration in fatal dose is not followed by acidosis

The transfusion experiment described, in which a diminished alkali reserve is seen in venous blood from a diphtheria toxin poisoned extremity, receiving normal arterial blood, offers a clue to the place of origin of the acidosis, and we believe the abnormal metabolism, of which acidosis is one of the evidences, occurs chiefly in muscle tissue

The explanation of the abnormal metabolism lies, we believe, in a diminished permeability of the damaged capillary wall, the diminution in the oxygen reaching the tissue cell resulting in a state of sub-oxidation. This belief is strengthened by the experiments with sodium nitrite and potassium cyanid, which show that a failure of the blood to give up oxygen, due to the formation of methemoglobin, or of the tissue to utilize available oxygen, will also cause acidosis

Finally, in application of our experimental facts, we believe that many cases of clinical acidosis may be explained by capillary injury. An acidosis, for example, may occur in many, if not all of the acute infectious diseases. Thus it has been observed in pneumonia,²² in diphtheria,²³ in scarlet fever²⁴ and in influenza²⁵. While little is known concerning the toxins in most of these infections, in one at least, diphtheria, the toxin is known and is the poison which, in our experiments, produced the most striking degree of acidosis obtained

Among other clinical cases which were accompanied by acidosis we find nephritis,²⁶ and cardiac disease²⁷. These conditions are considered by many to be part of a general vascular disease, the arteriocapillary fibrosis of Gull and Sutton, and looking at them from this standpoint, a relationship between the acidosis and capillary injury can easily be assumed

SUMMARY

1 Those poisons which have a wide spread action on capillaries, produce a marked degree of acidosis. Among these are diphtheria toxin, uranium and cantharidin

22 Palmer J Exper M **26** 495, 1917

23 Peters Brit M J **1** 10, 1918

24 Thomas Ibid **1** 274, 1919

25 Hachen and Isaacs J A M A **75** 1624 (Dec 11) 1920

26 Peabody Arch Int Med **16** 955 (Dec) 1915

27 Peabody Ibid **14** 23 (July) 1914

2 Renal injury is not an essential factor in the acidosis, since double nephrectomy fails to produce acidosis, and this condition can be brought about in nephrectomized animals

3 Those poisons which act selectively on the intestinal capillaries fail to produce acidosis, or if so, produce it to a minor degree

4 Marked injury to liver tissue does not cause acidosis

5 Evidence is submitted which points to muscle tissue as being one of the seats of acid formation

6 The assumption is made that the cause of the acidosis is a condition of suboxidation in the tissues, and experiments are reported showing that suboxidation produced by other means than capillary poisoning also causes acidosis

7 Capillary poisoning is suggested as an explanation of acidosis occurring in acute infectious diseases and some other clinical conditions

LOCAL DESENSITIZATION IN HYPERSENSITIVE INDIVIDUALS AND ITS BEARING ON THE PREVENTION OF HAY-FEVER[†]

GEORGE M MACKENZIE, M D, AND LOUIS B BALDWIN, M D
NEW YORK

Cutaneous reactions in hypersensitive individuals are of two quite distinct types. The reactions frequently observed in patients with hay-fever or bronchial asthma and those obtained after sensitization by foreign serum consist essentially of an urticaria-like lesion with a central wheal and a surrounding zone of erythema. Such reactions appear a few minutes after application of the test substance, usually reach their full development in half an hour or less, and fade out completely in one or two hours. The skin, at the site of the test then appears normal. There is no visible evidence of cell destruction. It appears to be essentially a vascular phenomenon with localized edema. Such reactions may often be obtained in hypersensitive individuals with extracts of pollens, animal hair, dandruff or feathers, with foreign serum, with many food proteins and occasionally with bacterial or other proteins.

These immediate urticaria-like reactions have little in common with the local cutaneous reactions produced by tuberculin, typhoidin, luetin and mallein. Here, the reaction does not develop for a number of hours, it is characterized by induration and persistent signs of inflammation, requires many days to fade out completely and clearly involves cell destruction. Zinsser¹ has recently shown that the local tuberculin reaction in the guinea-pig is independent of the development of a state of anaphylaxis, and it is highly probable that the same holds true for the reactions of this type produced by other substances of bacterial origin.

Although it has commonly been assumed that the immediate skin reactions with urticaria-like lesions are manifestations of anaphylaxis, it has not been demonstrated that the mechanism consists of an antigen-antibody reaction. Similar reactions may sometimes be obtained with nonantigenic substances, such as salicylates and quinin, and there are a few substances, notably histamin, morphin and pituitary extract which produce this type of reaction in normal individuals.

In an effort to determine the nature of these urticaria-like skin reactions, we have studied their exhaustibility in eight hypersensitive

[†] From the Medical Clinic of the Presbyterian Hospital, Columbia University.

¹ Zinsser, H. Proc Soc Exper Biol & Med **18** 123, 1921

individuals, six of whom had hay-fever or bronchial asthma, and two of whom were hypersensitive to horse serum following the intravenous administration of antipneumococcus Type I serum

TECHNIC

Both the cutaneous and intracutaneous methods of eliciting the skin reaction have been employed. The former was done by the familiar cut method, using a small, sharp scalpel to make a superficial incision about 2 mm long through the outer layers of the skin. To the cut is applied a drop of tenth normal sodium hydroxid and in this drop of alkali, a small amount of the test substance in powder form is rubbed into solution or an even suspension by means of a

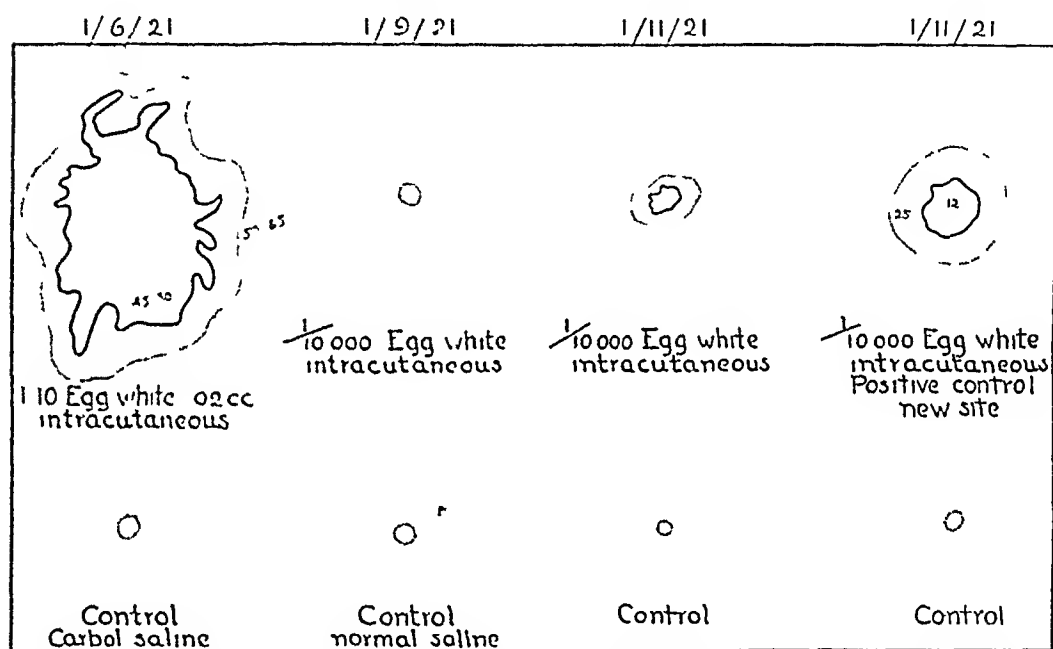


Fig 1—A McL No 48423 Male Age 6 Bronchial asthma and hypersensitiveness to egg. In this and the following figures the inner solid line figure represents the outline of the wheal and the outer dotted line represents the surrounding zone of erythema. The figures show the actual size in millimeters of the wheal and zone of erythema. Above the drawings, the date when each test was done is shown. Positive controls on new sites were done Jan 9, and Jan 11, 1921. They were of the same size and appearance. Only the latter is shown. The test on Jan 6, 1921 with a 1:10 dilution of egg white was followed by a general reaction so that in the subsequent tests, a weaker solution was used. It is seen that after an interval of three days, there was no reaction at the site of the initial test and that after five days, the reactivity had only partially returned.

sterile tooth pick. It was found that repeated applications of a drop of tenth normal sodium hydroxid to the same site frequently caused a small area of inflammation so that normal salt solution was substituted for the alkali. For the substances used, this gave specific reactions as strong as those obtained with alkali as solvent and did not cause any

irritation of the skin In the tests with egg white and horse serum, physiologic sodium chlorid solution containing 0.5 per cent phenol was used as a diluent

The intracutaneous tests were carried out by the usual technic, consisting of an injection of 0.02 c.c. into the superficial layers of the skin If properly done, a small injection wheal about 3 mm. in diameter should form when the solution is injected It was found that the latter method lends itself better to the demonstration of the exhaustion of the skin reaction than the cutaneous technic, although the reactivity may be abolished by either method

It was found that the exhaustion of reactivity is not readily demonstrable unless the application or injection of the test substance is repeated at short intervals Usually, we repeated the test as soon

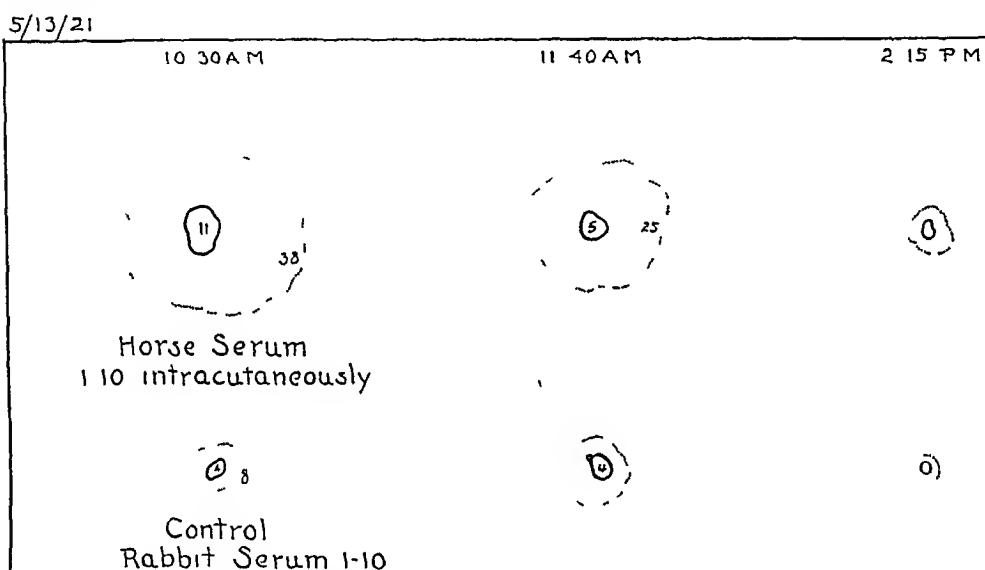


Fig 2—B S No 5901 Female Age 27 Bronchial asthma Hypersensitiveness to horse dander and horse serum This patient was spontaneously hypersensitive to horse serum though only to a moderate degree The local exhaustion of reactivity was accomplished by two intracutaneous reactions with 1:10 dilution of horse serum

as the preceding reaction had subsided, except when it was necessary to wait until the next day before completing the exhaustion The intervals in most cases between applications were therefore from one to three hours Some of the proteins used (almond, pea, oat, wheat) were obtained from a commercial firm, the egg white was prepared by making dilutions of fresh egg white in phenolized physiologic sodium chlorid solution, the ragweed extract was prepared by extracting the pollen with one hundredth normal sodium hydroxid, filtering through paper and a Mandler candle, the horse serum was obtained from the Department of Health of New York City

RESULTS

The semidiagrammatic drawings (Figs 1 to 8) illustrate eight of the thirty-six observations made on the eight patients. These results indicate that the specific reactivity of the skin may readily be abolished locally by repeatedly applying to the same site the substance to which the individual manifests cutaneous hypersensitiveness. The exhaustion has been accomplished with such biologically different substances as egg white, horse serum, extracts of ragweed and chicken feathers, the proteins of almond, pea, oat and wheat. Moreover, the reactivity is

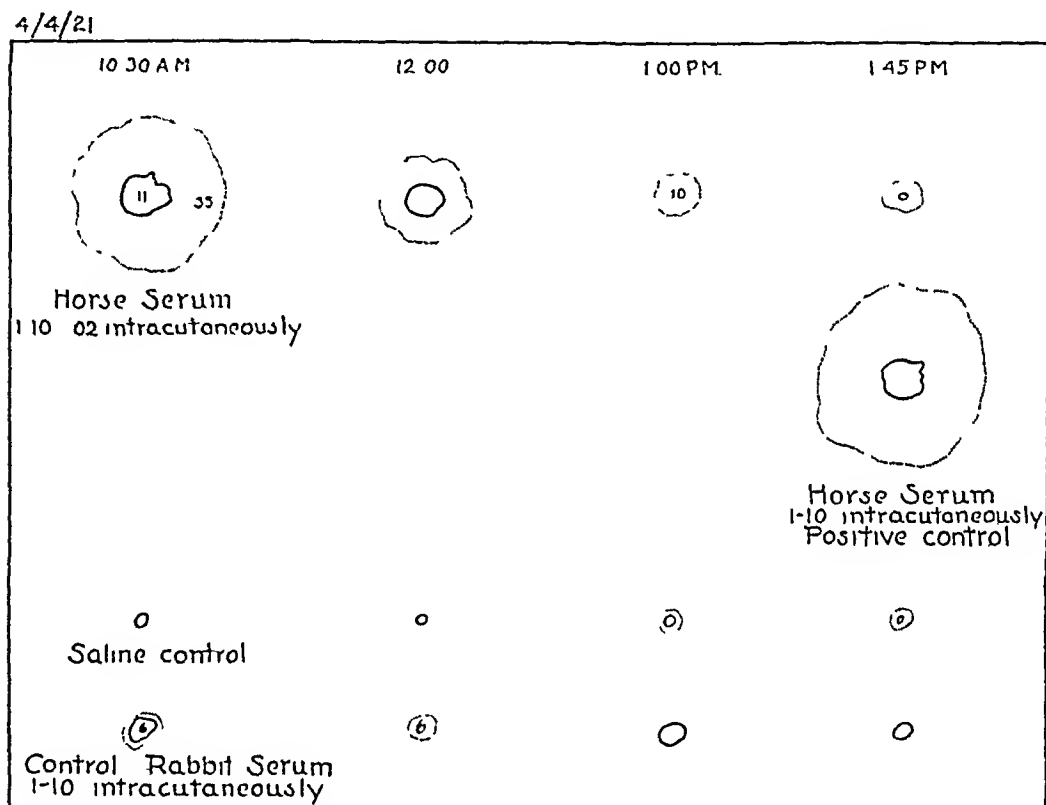


Fig 3—G R No 49327 Male Age 23 Patient had pneumococcus Type I pneumonia, was given 500 c c of antipneumococcus Type I serum. Serum disease developed on the seventh day after first serum treatment and persisted till the twelfth day. Cutaneous hypersensitiveness to horse serum developed on the fourteenth day after first serum. The figures represent the local exhaustion of reactivity to horse serum in a patient whose cutaneous hypersensitiveness was artificially produced. On admission to the hospital, the intracutaneous reaction with a 1:10 dilution of horse serum was negative.

more quickly abolished with concentrated than dilute solutions. Figure 1 shows that with a 1:10 dilution of egg white, a single intracutaneous test on a child 6 years old with bronchial asthma and hypersensitiveness to egg, completely abolished the skin reactivity at the site of the reaction. In Figure 2, the local exhaustion of reactivity in a patient spontaneously hypersensitive to horse serum is shown, and in Figure 3,

the same thing is shown for a patient artificially sensitized by serum treatment. In both these patients, the reactivity was quickly abolished.

In other trials, when substances presumably less antigenic were being tested or when weaker solutions were used, and the cutaneous technic was employed, from four to eight repetitions of the test were required to complete the exhaustion. In Figure 4, the exhaustion by means of the cutaneous technic is shown. In this instance, the reactivity persisted until the test had been repeated five times. The duration of the exhaustion has likewise been found to be dependent to some extent on the concentration of the protein solution and the method employed for producing the reaction. Thus, in Figure 1, it is shown

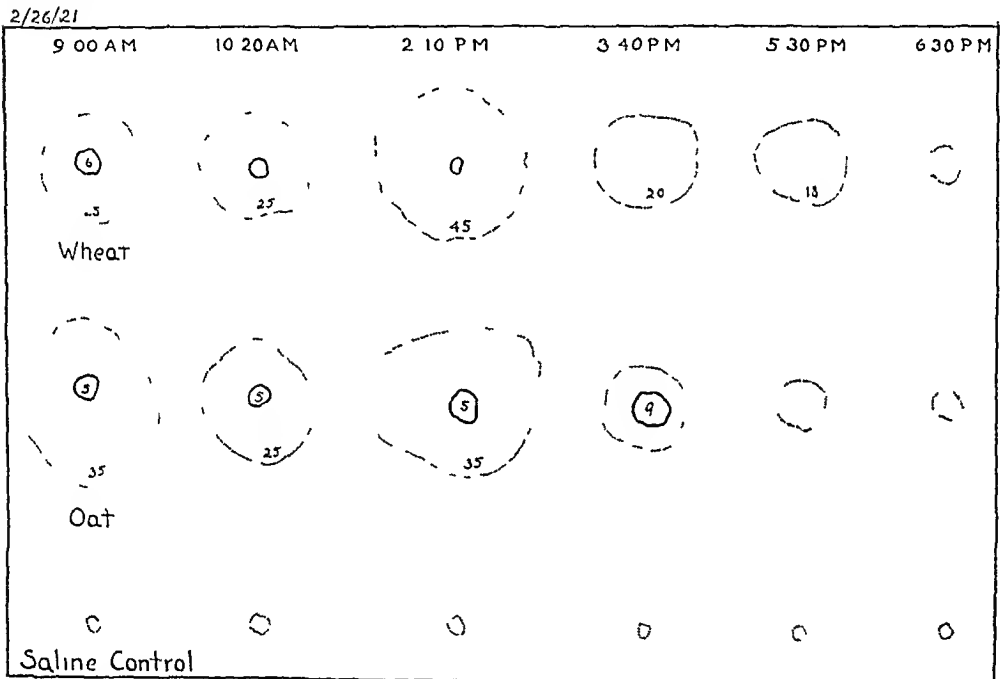


Fig 4—B Y No 49217 Male Age 33 Bronchial asthma, hypersensitivity to wheat, oat, rye, barley, rice. The local exhaustion of reactivity to wheat and oat is here shown to have been accomplished by the cutaneous technic after five applications.

that the reactivity of the child's skin at the site of the test was completely abolished for three days and partially abolished for five days. In other instances, with less concentrated solutions and substances whose antigenic properties are weaker, the exhaustion did not persist for more than twenty-four hours.

The extent of the area in which the reactivity is abolished is strictly limited to the site of the reaction. The area actually occupied by the wheal becomes completely exhausted, the area of the erythema partially so, and beyond this, the skin reacts as strongly as at a fresh site.

In order to determine the specificity of the exhaustion, we have made observations on three patients who gave clean-cut positive reactions to more than one substance. After simultaneously exhausting the reactivity of the skin at one site to one protein and at another site to a different protein, each protein was tested on the site exhausted by the other protein. Although the number of patients on whom it has been possible to study in this way the specificity of the exhaustion is too small to justify conclusions, the observations made thus far suggest that there is a strict specificity for substances biologically unrelated and something similar to group reactions for substances closely related biologically. Figure 5 shows the reactions of a patient

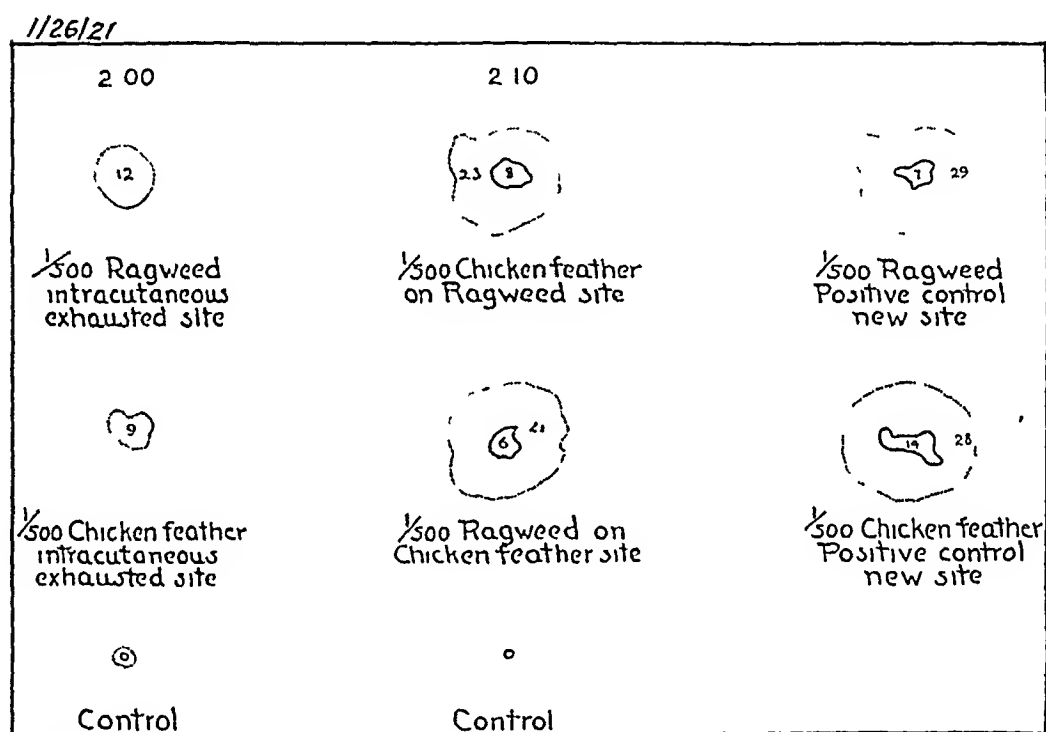


Fig 5—D H No 48838 Male Age 52 Bronchial asthma and hay fever Hypersensitiveness to ragweed and chicken feather. At the left are shown the reactions to ragweed and chicken feather after repeated intracutaneous tests at these sites had almost completely abolished the reactivity. Chicken feather was then tested on the exhausted ragweed site and ragweed on the exhausted chicken feather site. At the same time, each was injected at a fresh site. It is seen that the exhaustion is specific for both ragweed and chicken feather.

who manifested cutaneous hypersensitiveness to ragweed and chicken feather. It is seen that the exhausted ragweed site reacted as strongly to chicken feather as a fresh site and vice versa. Figure 6 in the same way illustrates the specificity of the exhaustion for the proteins of almond and pea. There is clearly no close biologic relation between ragweed and chicken feather nor between almond and pea. Figure 7, however, records the reactions of a patient hypersensitive to oat and wheat. It is seen here that the result suggests a group reaction. The

site which had been exhausted with wheat shows also a partial exhaustion of the reactivity to oat, for the exhausted wheat site reacted less strongly to oat than a fresh site

As a control series of observations, we have attempted in six patients to abolish the nonspecific cutaneous reactions to histamin. It is well known that histamin, either intracutaneously or by application to a small cut or scarification of the skin, produces an urticarial lesion quite similar in appearance to the reactions of individuals hypersensitive to pollen, animal dandruff, or feather extracts. Sollman² has studied the exhaustibility of the histamin skin reaction. With the scarification method, he found it inexhaustible. With the cut method and by intra-

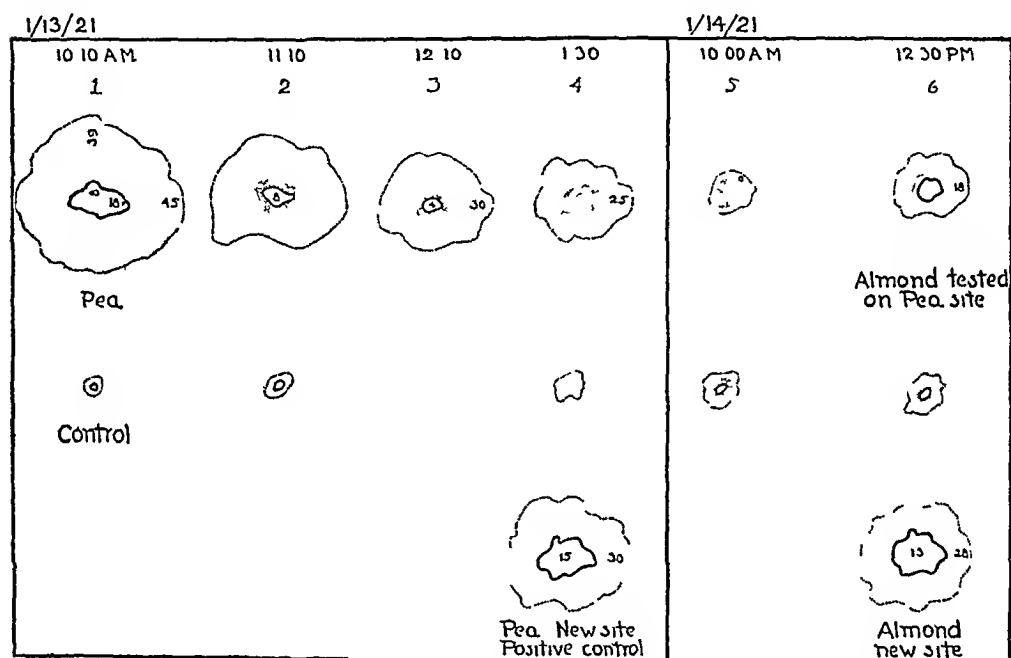


Fig 6—M K Female No 47992 Age 24 Bronchial asthma, urticaria, angioneurotic oedema. Hypersensitiveness to pea, buckwheat, cauliflower, lentil, almond. The first five figures in the upper row represent the successive stages of exhaustion of a skin area with pea protein by the cutaneous method. The sixth figure in the upper row represents the reaction to almond protein on the area in which reactivity to pea protein had been abolished. It is seen that this reaction is only a little less intense than the reaction produced by almond at a fresh site, shown in the lower right hand corner. The control reactions are shown in the second row. The reaction to pea at a fresh site, serving as a positive control, is also shown in the third row.

cutaneous injections of a 0.1 per cent solution, we have found that it is not only inexhaustible, but that the size and intensity of the reaction progressively increase with each subsequent application. Furthermore, exhaustion of the reactivity of a skin area with a specific antigen does not render this area refractory to histamin. In Figure 8 it is seen

² Sollmann, T. J. Pharmacol & Exper Ther **10** 147, 1917

that the site which had been exhausted for egg white gave a reaction to histamin which equals in intensity the histamin reaction at a fresh site

The nonspecific cutaneous reaction of pituitary extract has likewise been found to be inexhaustible With morphin sulphate, using a 3 per cent solution, the reaction, after four or five applications to the same site, has sometimes been inexhaustible, while at other times there was a partial loss of reactivity for a few hours

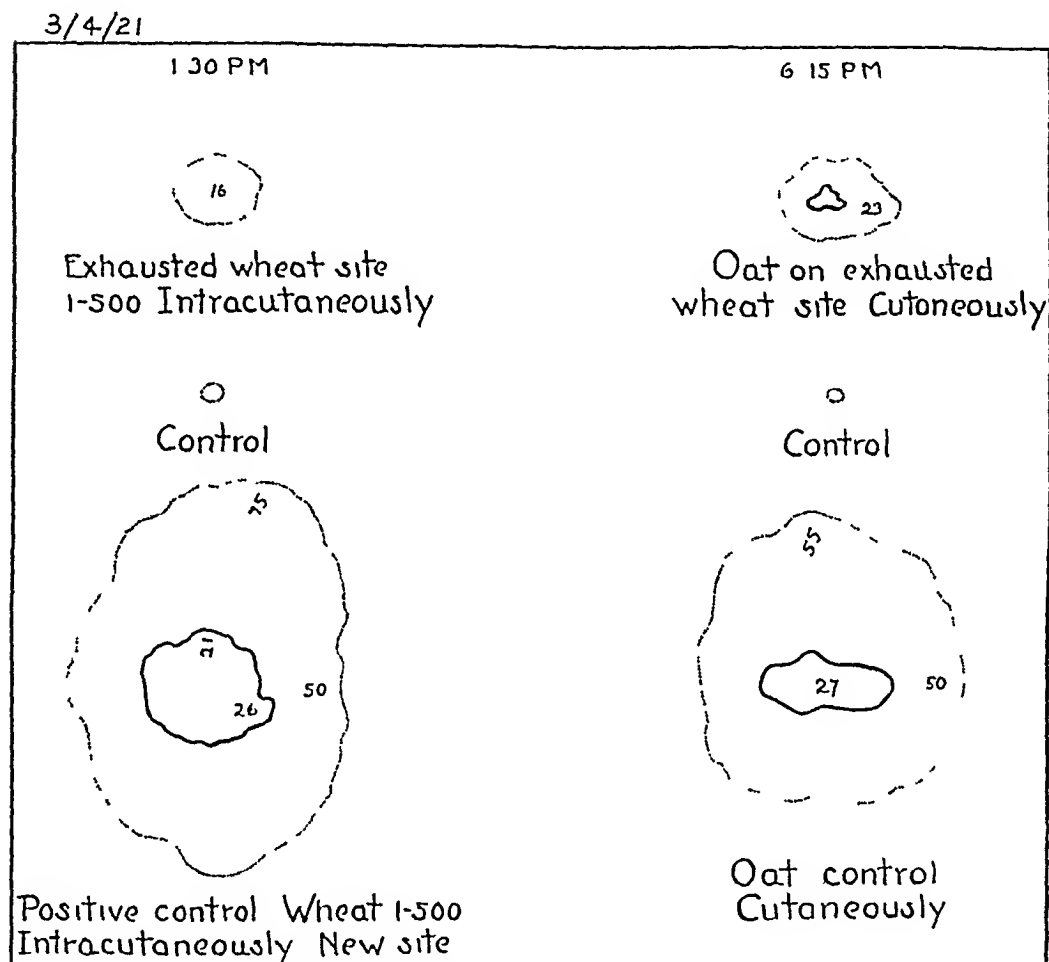


Fig 7—B Y No 49217 Male Age 33 Bronchial asthma, hypersensitive-ness to wheat, oat, rye, barley, rice Above at the left is shown the reaction by the intracutaneous technic of a 1 500 dilution of wheat after the reactivity had been almost completely abolished by eight previous tests at the same site during the preceding forty-eight hours Below this is shown the control reaction, using only the solvent, and below this, the reaction at a fresh site to the same solution of wheat protein The figures to the right show, above, the reaction to oat at the exhausted wheat site and, below, the reaction to oat at a fresh site It is seen that the exhaustion by wheat has effected a partial exhaustion for oat

DISCUSSION

The observations which we have described indicate that if the protein to which a patient manifests cutaneous hypersensitiveness is

repeatedly applied, either cutaneously or intracutaneously at short intervals to the same area of skin, the reactivity of the skin may be abolished locally. Furthermore, this exhaustion appears to be specific, for, if a patient gives positive skin reactions to two substances, biologically unrelated, the reactivity of an area of skin may be abolished for one substance without loss of reactivity for the other. Repeated efforts to abolish locally the reactivity of the skin to histamin have without

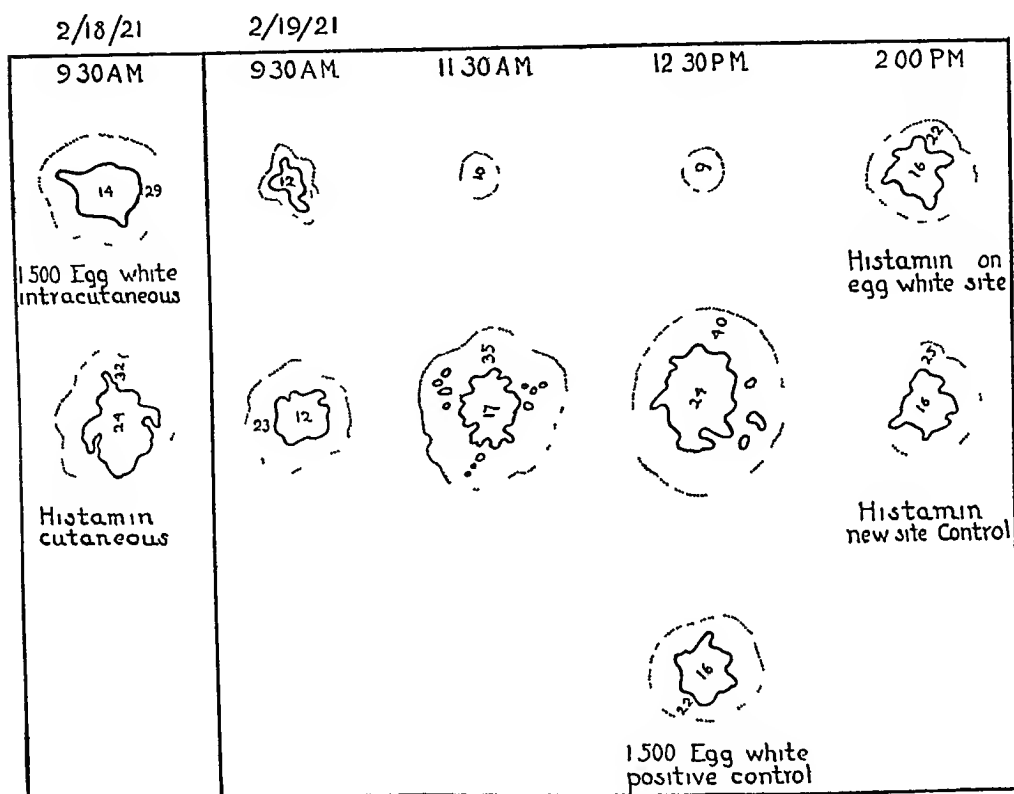


Fig 8—A McL No 48423 Male Age 6 Bronchial asthma, hypersensitivity to egg. The first four figures in the upper row represent the exhaustion of a skin area by the intracutaneous injection of a 1 500 dilution of egg white. It is seen that after the injection had been made twice, the reactivity was abolished for egg white. The figure furthest to the right in the upper row represents the reaction to histamin on the exhausted egg white site. It is seen that the histamin reaction is just as strong on this area as on a fresh site. The second row of figures illustrates typically the effect of applying histamin repeatedly to the same site. It is seen that the intensity of the reaction increases with each repetition after the first.

exception resulted not only in failure to accomplish the exhaustion, but in a demonstration that the nonspecific histamin reaction progressively increases in intensity with each subsequent application. To us it seems significant that the nonantigenic substance histamin produces a reaction which is inexhaustible, while the reactions produced by the antigenic substances which we have employed are readily exhaustible. Perhaps, the further observation that those substances

which are highly antigenic, such as egg albumin and horse serum, effect the exhaustion more promptly than less antigenic substances, such as pollen and feather extracts, is also significant. While the analogy between our results and the phenomena of antigen-antibody reactions is striking, we do not feel that one is justified as yet in interpreting the analogy as necessarily indicative of a similar mechanism. They do show, however, that the immediate urticaria-like cutaneous reactions of hypersensitive individuals behave much as one would expect them to behave if it were known that the mechanism consisted of an antigen-antibody reaction. The possibility naturally suggests itself that the local exhaustion of reactivity depends on a union of the test substance with something within the cells, and that as a result of this union, the constituent of the cells participating in the reaction is used up or becomes unavailable, that subsequently this intracellular reacting substance is released from the union or that it is reformed, enabling the cells to react again. At any rate, the phenomenon is certainly a local process and not a general one.

Owing to the long period of time required for such allergic skin reactions as that produced by tuberculin to develop and subside, it is not possible to test in the same way as we have done the local exhaustibility of cutaneous allergy of this type. Nor have we yet had an opportunity to try the exhaustibility of the immediate urticaria-like reactions occasionally produced by such nonantigenic substances as quinin and salicylates.

That exposure of tissue cells to contact with an antigenic substance may produce a local alteration of reactivity toward that antigen has recently been demonstrated by Besredka³ in his studies on local immunity. He has shown that administration of a formed antigen by mouth produces a local immunity in the gastro-intestinal tract apparently residing in the cells of the mucosa. An important factor in this immunity appears to be the decreased permeability of the mucosa for the antigen. Results of the same kind were obtained by Besredka in experiments in which the antigen was introduced intratracheally. In a similar way, our results indicate that in individuals with cutaneous hypersensitiveness, cellular reactivity may be altered locally by repeatedly bringing the antigen in contact with those cells.

With Besredka's results and our own in mind, we have commenced applying this principle therapeutically in hay-fever and allergic rhinitis from such substances as Florentine orris (a frequent constituent of face powders) and horse dander. From what we have observed in the treatment of these patients, it is clear that the local application of pollen, horse dander, or orris extracts brings about an alteration of

³ Besredka, A. *Ann de l'Inst Pasteur*, **33** 301, 557, 882, 1919, *ibid*, **34** 361, 1920

the reactivity of the nasal mucosa toward these substances with the result that the local tolerance is greatly increased. In some of our patients, after from four to six weeks of daily local application of the substance to which the individual is hypersensitive, the nasal mucosa has tolerated, without reaction, more than a thousand times the amount which at the outset caused marked symptoms. We intend to report later the therapeutic results in a group of patients treated in this way.

SUMMARY

1 Observations are reported which indicate that in individuals manifesting cutaneous hypersensitiveness, the reactivity of the skin may be abolished locally by repeatedly applying to the same skin area the substance to which the individual is hypersensitive.

2 The reactivity of the skin at the exhausted site may not return for three days, or perhaps longer.

3 The exhaustion appears to be specific.

4 The extent of the area of the exhaustion is strictly limited to the site of the reaction.

5 The nonspecific cutaneous reaction produced by the nonantigenic substance histamin is not only inexhaustible but progressively increases with each repetition of the application to the same site.

6 The possibility that the results reported indicate a genuine local desensitization is discussed.

7 The bearing of the results on the treatment of hay-fever and other forms of allergic rhinitis is discussed.

PRIMARY CARCINOMA OF THE LUNGS *

T S MOISE, M D

NEW HAVEN, CONN

CONTENTS

- I Introduction
- II General Considerations
 - (A) Historical
 - (B) Incidence
 - (C) Clinical Characteristics
 - (D) Metastases
 - (E) Anatomy and Embryology
- III Classification
 - (A) According to Gross Anatomy
 - (B) According to Histological Anatomy
 - (C) According to Histogenesis
- IV Relation of Irritation to Neoplastic Growth
- V Case Reports
- VI Discussion
 - (A) Incidence
 - (B) Clinical Characteristics
 - (C) Metastases
 - (D) Gross and Histologic Features
 - (E) Histogenesis
 - (F) Relation of Irritation to Neoplastic Growth
- VII Conclusions

INTRODUCTION

The relative rarity of primary carcinoma of the lungs and bronchi, the difficulty of making a clinical diagnosis and the comparative inaccessibility in regard to surgical procedures, have tended to limit the practical interest in these tumors. In spite of these limitations, there is a very extensive literature on the subject which suggests, at least, a wide scientific interest in this group of neoplasms.

This paper is based on the study of five cases of primary carcinoma of the lungs. The object will be to give a brief résumé of the pathology of carcinoma of the lungs, with some emphasis on the problem of histogenesis and on the possible etiologic importance of acute and chronic irritation.

* From Brady Laboratory of Pathology and Bacteriology, Yale University School of Medicine

GENERAL CONSIDERATIONS

Historical—The earliest published record of a pulmonary carcinoma is Morgagni's¹ description of a necropsy in which the only finding was an "ulcus cancrum" of the right lung. The second noteworthy contribution to this subject was Bayle's² clinical and pathologic study of three cases which he described under the name of "phthisie cancreuse." He described the co-existence of cancer and tuberculosis, but did not give a clear idea of the difference between the two conditions. He believed that tuberculosis was the result of an acid and cancer of an alkali. He correctly stated that pulmonary neoplasms may exist without symptoms for a relatively long time. The phrase "phthisie cancreuse" was the subject of a prolonged discussion, and led to great confusion between cancer and tuberculosis of the lungs. Laennec³ described "encephaloid" tumors of the lungs, and many authors of this period used the terms "fungus hematodes" and "fungus of the lungs" to describe malignant pulmonary tumors.

The great advance in the study of lung tumors came with the development of cellular pathology under the influence of Virchow. Since this time the question of the histogenesis of these tumors has absorbed the interest of many investigators. Three sites of origin for pulmonary carcinoma have usually been given, (1) the bronchial mucous membrane, (2) the bronchial mucous glands, and (3) the alveolar epithelium. The fact that there have been great differences of opinion concerning the characteristics of these three types of tumor, indicates the difficulties of coming to a definite conclusion in an individual case. Among the earlier histologic studies is that of Langhans⁴ in which he described a case generally accepted as arising from the bronchial mucous glands. The subsequent development of the subject may be outlined briefly by noting a few of the numerous attempts to collect the reported cases. Reinhard⁵ collected twenty-seven cases. Wolf⁶ reported thirty-one cases from the Dresden Pathological Institute. A year later Paessler⁷ was able to find seventy cases in the

1 Morgagni, J. B. De sedibus et causis morborum per Anatomen indagatis (quoted by Wolff, J.¹⁰) 1708

2 Bayle, G. L. Recherches sur la phthisie pulmonaire, Paris, 1810 (Quoted by Wolff, J.¹⁰)

3 Laennec, R. T. H. De l'auscultation mediate, on traite du diagnostic des maladies des poudrons et due coeur, Paris, 1818 (Quoted by Wolff, J.¹⁰)

4 Langhans, T. Primarer Krebs der Trachea und Bronchien, Virchows Arch f path Anat **53** 470, 1871

5 Reinhard, W. Der Primare Lungenkrebs, Arch f Heilk **19** 369, 1878 (Quoted by Paessler⁷)

6 Wolf, K. Der Primare Lungenkrebs, Fortschr d med **13** 725, 765, 1895

7 Paessler, H. Ueber das Primare Carcinom der Lunge, Virchows Arch f path Anat **145** 191, 1896

literature and added four new cases. Sehrt⁸ reviewed 177 published cases. Adler⁹ collected a total of 374 cases and since that time Scott and Forman¹⁰ (1916) have found reports of 120 additional cases.

It may be of interest to review briefly some of the data which these reports have brought together.

Incidence—Composite statistics show that primary malignant tumors of the lungs occur in about 1 per cent of all carcinomas and in about 0.36 per cent of all necropsies. The older statistics give a smaller percentage. Reinhard⁵ reported 8,716 necropsies at the Dresden City Hospital in which there were five primary lung tumors in a total of 545 cases of carcinoma. Fuchs¹¹ (Munich) found eight cases of primary lung cancer in 12,307 necropsies. The percentages quoted above are based on the following reports. Among 16,578 cancer necropsies compiled by Adler,⁹ there are 168 primary lung tumors. Weller¹² quotes statistics showing forty cases in a total of 11,903 necropsies. There is, however, a growing opinion that primary malignant tumors of the lungs are more common than these statistics indicate. In spite of the prevalence of this opinion, these figures are corroborated in a comparatively recent paper by Scott and Forman,¹⁰ who report three primary bronchial tumors in a series of 302 deaths from malignant disease.

Age—The following table from Adler⁹ gives the age incidence in 356 cases.

TABLE 1—AGE INCIDENCE OF LUNG CANCER

Age	No Cases
1-10	0
10-20	6
20-30	10
30-40	30
40-50	78
50-60	113
60-70	94
70-80	23
80-90	2
Age not stated	18
Total	374

8 Sehrt, E. Beiträge zur Kenntnis des primären lungen Carcinom, Inaug Diss., Leipzig, 1904.

9 Adler, I. Primary Malignant Growths of the Lungs and Bronchi, New York, 1912.

10 Scott, E., and Forman, J. Primary Carcinoma of the Lungs, Med Rec 90 452, 1916.

11 Fuchs, E. Beiträge zur Kenntnis der geschwulstbildungen in der Lunge, Inaug Diss., München, 1886 (Quoted by Watsuji¹⁷).

12 Weller, C. V. Primary Carcinoma of the Larger Bronchi, Arch Int Med 11 314 (Feb) 1913.

Clinical Characteristics—Lung tumors are only rarely diagnosed clinically. The difficulties of diagnosis are obvious, and the reason for failure in a large percentage of the cases is evident when we consider that the symptoms on which a diagnosis might be made are the result of extensive damage to the pulmonary parenchyma or bronchial obstruction, producing interference with the respiratory function. Furthermore, it is clear that, generally speaking, such signs and symptoms do not serve to differentiate pulmonary tumors from many other intrathoracic conditions.

With the purpose of studying the clinical characteristics of primary bronchial carcinoma, Weller has collected ninety cases in which the diagnosis was based on microscopic examination at necropsy and in which there was good evidence of bronchial origin. He divides these tumors into three clinical groups: (1) small tumors producing no clinical symptoms, (2) tumors in which the dominant symptoms are produced by cerebral or other metastases, and (3) those in which the patient is first seen in a moribund condition, to these groups may be added, (4) those showing symptoms and signs of a more or less extensive pulmonary involvement with or without evidence of metastases.

In the series of ninety cases collected by Weller,¹² a clinical diagnosis was made in ten instances. He has outlined the chief symptoms as follows: (1) cough, (2) dyspnea, usually progressive in character, (3) hemoptysis, varying from blood streaks to bright red blood (the "black currant jelly" or "raspberry jelly" sputum long considered characteristic of these tumors is rarely mentioned), (4) pain, usually referred to chest or shoulder and arm on the affected side, and (5) symptoms due to pressure on important mediastinal structures.

Weller emphasizes the diagnostic importance of the absence of fever and night sweats, of bronchoscopic and radiographic examinations, and of histologic examinations of excised lymph nodes.

Metastases—Widespread metastases are relatively common findings in primary lung tumors. An analysis of 327 cases in which the distribution is definitely stated (Adler,⁹ Lambert¹³) shows thirty-three cases in which there were absolutely no metastases and sixty-three cases in which metastases were confined to the thoracic cavity. The remaining cases showed a high percentage of generalized metastases. On this basis carcinoma of the lungs may roughly be divided into three classes: (1) those showing no metastases, (2) those in which secondary foci were limited to the thorax, and (3) those showing generalized metastases. The possible pathways through which extension may take place are obviously the lymphatics, the blood vessels and the air spaces.

¹³ Lambert, R. A. Personal communication.

In some cases of generalized carcinomatosis, almost every organ and part of the body may be involved, but the organs most commonly the seat of secondary tumors are, in approximate order of frequency, the regional lymph nodes, liver, kidneys, lungs, pericardium, abdominal lymph nodes, pleura, brain, suprarenals, bones, cervical lymph nodes and heart

Anatomy and Embryology—Before discussing the classification of these tumors, a brief review of the developmental stages, the normal anatomy, and the lymphatic drainage of the lungs is important

The embryology may be recalled by quoting briefly from Flint¹⁴

In the organogenesis of the lungs, we have the stem and main bronchi consisting of simple tubes lined by a double layer of epithelium, the inner of which is columnar, while the outer is composed of small polygonal cells

Later, the mucosa is thrown into a series of longitudinal folds, while from the cuticular border of the inner row of cells, cilia develop. From the bottom of the crypt-like invaginations formed by the longitudinal folds of epithelium, glands begin to grow into the submucosa, which sometimes pass between the developing muscle bundles into the deeper layers of this coat. As this process takes place there is a differentiation of some of the epithelium into goblet cells, a process, which one also observes in the glands, giving rise to a series of submucous glands with partly serous and partly mucous cells.

As we follow the bronchi peripheralward, they become simpler and essentially younger in structure and yet develop their adult characteristics in precisely the same way. The epithelium soon becomes single layered and of a columnar type as the periphery is reached. Finally it takes on a distinct, flat, cubical form. And finally, after birth there is a dilatation of the lobules and further flattening occurs.

The mucous membrane of the trachea and the larger bronchi is composed of three layers, an outer cylindrical layer of ciliated and goblet cells, a middle layer of spindle shaped cells, and an inner layer of wedge shaped, formative cells which rests on the basement membrane. The trachea and the larger bronchi are provided with numerous small mucous glands which are situated within the submucosa, within the fibrous layer and between the cartilaginous rings. Within the lungs the cartilaginous rings occur in bronchioles measuring as small as 1 mm in diameter. Mucous glands are found throughout the greater part of the bronchial tree. They are most numerous in the larger bronchi and they are rarely, if ever, found in bronchioles measuring less than 1 mm in diameter. The mucous membrane, even in the smaller bronchioles, retains its ciliated columnar character, but consists of a single layer of cells. Finally, the bronchioles lose their cylindrical character and there is a transition from the columnar epithelium to a nonciliated layer of cells. At the termination of the lobular bronchioles and within the alveoli, the epithelium is of the flat pavement variety.

14 Flint, J. M. The Development of the Lungs, *Am J Anat* 6 1, 1906

The lymphatics of the lung may be divided into a superficial and a deep set (Miller¹⁵) The former comprise the pleural lymphatics, the latter comprise those situated in the peribronchial and perivascular areas, as well as those found in the interlobular connective tissue septa.

The bronchial, the arterial and the venous lymphatics anastomose freely but form separate plexuses. The larger bronchi have two sets of lymphatics which enclose the cartilages but are connected by numerous intercartilaginous anastomosing vessels. These lymphatics become smaller and less numerous, finally terminating at the distal end of the lobular bronchioles. There are no lymphatics distal to the lobular bronchioles, that is, in the walls of the atria or alveoli. The periarterial plexus also becomes gradually smaller, and the smaller arterioles are frequently accompanied by only a single lymphatic. The common anastomoses between the bronchial and arterial lymphatics are found at the points of division of the bronchi and at the distal ends of the lobular bronchioles. The pulmonary veins arise from the pleura, the alveolar walls, the distal ends of the lobular bronchioles, and from the points of division of the bronchi, lymphatic channels arise with the veins from all of these locations except the alveolar walls, where there are no lymphatics. The complexity and size of these lymphatics increase with the size of the veins. The lymphatics accompanying the veins from the pleura and from the bronchi anastomose, respectively, with the pleural and bronchial lymphatic plexuses. The most constant valves are situated in the interlobular septa, at the junction of the venous and pleural lymphatics. These valves point toward the pleura. There are no other anastomoses between the pleural and the pulmonary lymphatics. The pleural lymphatics form a dense complicated plexus with numerous valves. The anastomoses are very numerous and the valves offer no resistance to the flow in any direction. The normal flow is over the surface of the lung toward the hilum. The normal flow of the pulmonary lymphatics is likewise toward the hilum. Throughout the lungs, the lymphatics generally have no valves, however, they are sometimes present in the large perivascular channels.

CLASSIFICATION

Primary carcinoma of the lungs and bronchi may be classified according to (1) gross anatomy, (2) histology and (3) histogenesis.

Classification According to Gross Anatomy—The gross anatomic appearance of lung tumors is very variable and there are no well defined gross types. A nodular form, an infiltrating form, and a miliary carcinosis have been described, and these descriptive terms are frequently used in classification, although they are obviously appli-

15 Miller, W. S. Studies in Tuberculous Infection. III. The Lymphatics and Lymph Flow in the Human Lung, *Am Rev Tuberc* 3 193, 1919.

cable rather to stages in the growth of a tumor than to distinct types of tumor. The nodular form includes rare cases of a single nodule or a few small nodules in the pulmonary parenchyma, or localized papillary tumors arising in the walls of tuberculous or bronchiectatic cavities and are only discovered incidentally at necropsy. The infiltrating form is the usual finding, as the majority of cases are seen in an advanced stage when there is extensive invasion or infiltration of the lung. In these tumors the most significant gross finding is that the tumor is confined to, or is much more extensive in, one lung, while secondary tumors are frequently distributed widely throughout both lungs. In advanced stages of the primary tumors, the distribution of the cancerous invasion within the lung is quite uniform. The bronchi and blood vessels are encircled by bands of tumor, which become more and more conspicuous as the hilum is approached. These peribronchial tumor masses may compress the larger bronchi with resulting total or partial occlusion leading to atelectasis and bronchiectasis. A large mass of carcinomatous tracheobronchial lymph nodes is frequently found filling the angle between the main bronchi. The hilar nodes on the opposite side are not infrequently involved, but the opposite lung itself is relatively free from tumor nodules. The cases described as a milary carcinosis are said to resemble milary tuberculosis, except that the nodules are somewhat larger, more translucent and whiter in color, and are situated only along the course of the lymphatic channels. This finding is apparently indicative of an early widespread lymphatic invasion of the lung.

Classification According to Histologic Anatomy—In fifty-five cases reviewed by Weller¹² the structural character of the neoplasm was spoken of as alveolar, acinar, or adenomatous in forty-five, medullary in five, scirrhous in three and simplex in two. According to the cell type these cases were described as squamous in twenty-five, cylindrical in eighteen, polymorphous in ten, round or oval in two and gland and cuboidal in one each. Notwithstanding this variety, it is probable that all cases can be placed in three types, (1) cylindrical cell, (2) polymorphous cell and (3) squamous cell.

The largest number of cases are placed in the cylindrical cell group by the majority of writers (Langhans,⁴ Wolf,⁶ Paessler,⁷ Adler,⁹ et al). There are great variations in the histologic characteristics of these tumors, and not infrequently several different types of cell are found in the same tumor. Among the types are cylindrical cells with and without cilia (Horn¹⁰), cuboidal cells, polymorphous cells, and mucous secreting cells. The predominating cell is, in most cases, of the

16 Horn, O. Ein Fall von primären Adenocarcinom der Lunge mit flimmerndem Zylinderepithel, *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* 189 414, 1907.

cylindrical or cuboidal type The polymorphous cell tumors include those cases in which the predominating cell is of an undifferentiated type and cannot be included in the cylindrical or the squamous cell groups The squamous cell tumors are said by many investigators to be an infrequent type, but Watsuji¹⁷ places 32.2 per cent in this category This difference of opinion may be due to a difference in the interpretation of what constitutes a squamous cell By some writers only those cells are so classed which tend to undergo keratinization Ernst¹⁸ insists on this qualification and says that the presence of flattened cells alone or even epithelial whorls is not sufficient basis for calling a tumor squamous celled, since other types of cell may undergo such physical modification From a critical review of the reported cases it is clear that while tumors composed of flattened cells are fairly common, making up possibly a third of the total, definite prickly cell keratinizing growths constitute a relatively small group The dividing line between the two groups is, however, not sharp and this renders the classification of certain cases very difficult

Classification According to Histogenesis—Three sites of origin for primary carcinoma of the lungs are usually given (1) the bronchial mucous membrane, (2) the bronchial mucous glands, and (3) the alveolar epithelium The first two are generally spoken of as primary bronchial and the third as primary pulmonary tumors

The primary bronchial tumors comprise the majority of cases and, according to many authors, the primary nodules are usually situated in the larger bronchi (Paessler,⁷ Langhans,⁴ Adler,⁹ Wolff¹⁰)

The discussions of the histogenesis of lung tumors and of metaplasia of the epithelium of the lungs are inextricably interwoven, and accordingly an understanding of the changes in the lining epithelium of the respiratory tract, which may be broadly grouped under the term metaplasia, is essential While there has been a prolonged discussion of the question of metaplasia, it is now generally conceded that this term does not imply a direct change of one cell into a cell of a different order, but a reversion of the formative cells of an epithelial tissue, so that their specificity is lost at the expense of regaining the powers of differentiation possessed by embryonic epithelial cells The term false metaplasia and pseudo-metaplasia may be used to describe changes in form alone or a histological accommodation, as when the cylindrical cell lining of a cyst becomes flattened by pressure, undergoing mechanical changes only, and changes in the type of epithelium, really a

17 Watsuji, S. Beiträge zur Kenntnis des primären Hornkrebses der Lunge, Ztschr. f. Krebsforschung 1: 445, 1904

18 Ernst, P. Ein verhornender Platten-epithelkrebs des Bronchus. Metaplasia oder aberration, Beitr. z. path. Anat. u. z. allg. Path. 20: 155, 1896

19 Wolff, J. Die Lehre von der Krebskrankheit, Jena, 1911, Part 2, p. 803

substitution process, as is seen in the hornification of the cervix by the ingrowth of vaginal epithelium (Borst ²⁰)

By a study of cases of bronchopneumonia showing multiple foci of squamous cell metaplasia of the bronchial lining epithelium, Haythorn ²¹ has attempted to determine the mechanism through which these changes have occurred. He investigated each layer of the mucous membrane separately and concluded that the absence or the rarity of columnar cells and goblet cells precludes the probability of their taking part in the process, and concluded that the regeneration and transformation must take place from the cells of the formative layer. He stated, furthermore, that there was in no instance an example of stratified squamous epithelium resting on a well developed basement membrane. His conclusions included the views, that the metaplastic cells are produced by the formative layer of the mucous membrane, that the finding of multiple foci of squamous cells is against the idea of embryonic rests, and that there is suggestive evidence of a link between injury to the basement membrane, metaplasia, and the formation of new growths.

The proliferative changes in the epithelium of the lungs secondary to inflammatory reactions have been studied by many investigators (Kitamura, ²² McKenzie, ²³ Haythorn ²¹). The presence of squamous epithelium in tuberculous cavities and in the bronchi leading to them is a common observation. There are also many observations of similar changes distributed widely throughout the lungs in chronic or organizing bronchopneumonia, and McKenzie ²³ has described these changes in acute bronchopneumonia. It might be noted in this connection, that a change from cylindrical epithelium to squamous epithelium associated with chronic inflammatory conditions is also seen in the gall bladder, pancreas, and in other organs. The change of alveolar epithelium in scars and of regenerating alveolar epithelium from a flat, pavement-like cell to a cuboidal or columnar type is important in understanding the histogenesis of lung tumors. The many other changes in epithelial cells associated with inflammatory processes are too well known to require a detailed description. Some of these changes, along with the striking pictures of epithelial regeneration seen in the recent influenza epidemic, will be referred to later.

20 Borst, M. Das pathologische Wachstum, Aschoff, L., Pathologische Anatomie, Jena, 1 41, 1919

21 Haythorn, S. R. On the Metaplasia of Bronchial Epithelium, J. M. Research 26 523, 1912

22 Kitamura, S. Ueber secundäre Veränderungen der Bronchien und einige Bemerkungen über die Frage der Metaplasie, Virchows Arch f path Anat 190 163, 1907

23 McKenzie, I. Epithelmetaplasie bei Bronchopneumonie, Virchows Arch f path Anat 190 350, 1907

Carcinoma Arising from the Bronchial Mucous Membrane—These tumors are described as producing extensive changes in the bronchial mucosa advancing along the walls of the bronchi and septa, but seldom showing a diffuse involvement of the pulmonary parenchyma (Reinhard,⁵ Ewing²⁴) The bronchi may be filled with papillary projections over a wide area producing a thickening of the wall and invading the parenchyma Obstruction to the bronchi and resulting bronchiectasis and atelectasis are not infrequently described Small tumors arising in the walls of tuberculous cavities or appearing as nodular projections into the lumen of a bronchus, also undoubtedly take their origin from the epithelium of the bronchi

The histologic characteristics of tumors arising from the bronchial mucosa have been variously described Adler⁹ says that they are of alveolar structure with polymorphous and polyhedral cells, for the most part flat, but that sometimes varying numbers of cylindrical cells are intermingled, while Ewing²⁴ states that cylindrical cells may be found in all forms of lung cancer, but that the presence of high cylindrical cells and their persistence in metastases is indicative of origin from the bronchial mucosa He states, furthermore, that carcinoma may also arise from metaplastic bronchial epithelium giving a tumor of the squamous cell type

The squamous cell tumors discussed in a preceding paragraph on histologic classification were first described as arising from the alveolar epithelium (Perls,²⁵ Fuchs,¹¹ Japha,²⁶ Grunwald,²⁷ Wolf,⁶ Domeny,²⁸ Edlavitch²⁹), but the embryology of the pulmonary epithelium, the cubical form taken by cells of isolated alveoli in fibrous scars, and the appearance of regenerating alveolar epithelium, are against this view

It is now generally conceded that the squamous cell tumors arise from the bronchial mucous membrane (Wolf,⁶ Sehart,⁸ Ernst,¹⁸ Watsuji,¹⁷ Friedlander,³⁰ Hermann³¹) Two explanations of this occurrence have been proposed, (1) metaplasia of the normal epithelium, and (2) the embryonic displacement of islands of squamous cells The

24 Ewing, J Neoplastic Diseases, Philadelphia, 1919, p 785

25 Perls, M Zur Kasuistik des Lungenkrebses, Virchows Arch f path Anat **56** 437, 1872

26 Japha, A F Ueber primaren Lungenkrebs, Inaug Diss, Berlin, 1892 (Quoted by Watsuji¹⁷)

27 Grunwald, L Ein Fall von primaren Plasterzellenkrebs der Lunge, Munchen med Wchnschr **36** 548, 1889

28 Domeny, P Zur Kenntniss des Lungen carcinoms, Ztschr f Heilk **23** 407, 1902

29 Edlavitch, B M Primary Carcinoma of the Lung, J A M A **59** 181 (July 20) 1912

30 Friedlander, C Kankroid in einer Lungenkaverne, Fortschr d med **3** 307, 1885

31 Hermann, M Ein Fall von primaren Cancroid der Lunge, Ztschr f Krebsforsch **13** 446, 1913

consensus of opinion is in favor of the first explanation. This is chiefly because of the fact that a conversion of the normal columnar cell lining into a layer of squamous cells in association with chronic inflammatory changes in the bronchi, is not infrequently seen. The second explanation was proposed by Siegert³² who called attention to the fact that the anlage of the respiratory tract is a ventral outgrowth from the foregut, and suggested that squamous celled tumors of the lungs may develop from aberrant oesophageal epithelium. Peritz³³ called attention to the incorrectness of this idea in that during early embryonic life the esophagus is lined with ciliated cylindrical epithelium.

Carcinoma Arising from the Bronchial Mucous Glands—A high percentage of the cylindrical cell tumors are attributed to this origin (Fuchs,¹¹ Kretschmar,³⁴ Wolf,⁶ Langhans⁴). This view is apparently based on changes in the appearance of the bronchial mucous glands, on the glandular architecture of the tumors, and on the tendency of the tumor cells to secrete mucus. The tumor mass in typical cases is limited to the bronchial wall and the tissues immediately adjacent. The mucosa of the bronchus may be intact.

A classical example of this type was reported by Langhans⁴. There was extensive involvement of the bronchial tree. Just above and below the bifurcation of the trachea, the wall was thickened and elevated without ulceration of the overlying mucosa, but with extension into the peritracheal tissue. In the smaller bronchi there were also nodular elevations and a uniform infiltration of the wall, producing a narrowing of the lumen. Microscopically, alveoli, solid nests and strands of cells in a connective tissue background, were seen extending from the mucosa to the peribronchial tissue. These cell groups resembled closely the bronchial mucous glands. Langhans described a complicated glandular architecture and believed that different areas represented steps in the transition from the normal bronchial mucous gland to the cancerous alveoli. With this interpretation he described (a) enlargement of the glands up to eight and ten times the normal size with a single layer or many layers of lining cells to complete obliteration of the lumen, (b) extreme irregularity in the shape and size of the glands, (c) loss of the basement membrane, (d) numerous intercommunications between these large, irregular, glandlike spaces.

The conclusion that this tumor arose in the bronchial mucous glands was based on the absence of gross involvement of the bronchial lining epithelium, the extensive involvement of the bronchial wall, and the histologic resemblance to the normal glands.

32 Siegert, F. Ueber Primäre Geschwulste der unteren Luftwege, Virchows Arch f. path. Anat. **129** 413, 1892.

33 Peritz, G. Ueber Brusthöhlen Geschwulste, Inaug. Diss., Berlin, 1896 (Quoted by Watsugi¹⁷).

Carcinoma Arising from the Alveolar Epithelium—Some authors have described squamous cell tumors as having this origin. However, a study of the development of the alveolar epithelium, which in the early stages is cuboidal or columnar, and, similarly, observations upon regenerating alveolar epithelium which is also cylindrical or cuboidal, are against this view. The consensus of opinion is that these tumors arising from the alveoli are not composed of squamous cells but of cells of the cylindrical or cuboidal variety. Kretschmar³⁴ has described a case which has been accepted generally by later investigators as of undoubted alveolar origin. In this case, there was extensive infiltration of the lung tissue, giving the gross appearance of an organizing bronchopneumonia. The tumor was definitely alveolar in structure, consisting of large alveoli lined with a single layer or many layers of high cylindrical cells. Some alveoli showed infoldings and papillary projections into the lumina, while in other places there was a more intricate branching structure. There were also extensive areas of necrosis with only faintly visible alveolar outlines. At the periphery there were a few alveoli showing a single layer of low cuboidal epithelium or several layers piled up to form small projections into the lumina. Kretschmar³⁴ interpreted this picture as the beginning of the process. In other places there were many irregularly arranged, large polygonal cells with very large nuclei.

The bronchi were filled with mucus and cellular detritus. The epithelial lining and membrane propria were for the most part intact. The bronchial mucous glands were hyperplastic. The submucosa contained nests of irregularly round, oval, or polygonal cells, having no connection with the mucous glands.

The diagnosis of alveolar origin of this tumor was based on the extensive involvement of the pulmonary parenchyma, the relatively slight involvement of the bronchial lining epithelium, the absence of any demonstrable connection with the bronchial mucous glands, and the resemblance to regenerating alveolar epithelium.

In discussing the alveolar origin of lung tumors, Paessler⁷ stated that forty-seven of fifty-four reported alveolar tumors, or 87 per cent, were apparently of bronchial origin. Furthermore, he concluded that the morphologic characteristics of lung tumors are so variable that it is impossible to determine their histogenesis in the majority of cases.

RELATION OF IRRITATION TO NEOPLASTIC GROWTH

Chronic irritation of the respiratory tract has long been considered an important factor in the causation of lung tumors. Adler⁹ calls

³⁴ Kretschmar, W. Ueber das primäre Bronchial und Lungencarcinom, Inaug. Diss., Leipzig, 1904.

attention to the much higher percentage of cases in men than in women, and suggests that this difference may be attributed to irritation from tobacco smoking. In many instances a preceding history of chronic bronchitis and bronchopneumonia is obtained, and many authors have called attention to this association. The marked epithelial overgrowth in bronchitis and bronchopneumonia, as well as the changes in the epithelium frequently seen in fibrotic areas, is suggestive, and Menetrier³⁵ and Ribbert³⁶ have suggested that lung tumors may arise in these areas.

The greatest emphasis has been placed on the association between tuberculosis and cancer of the lungs. Bayle² first described co-existent cancer and tuberculosis, and subsequently many observers have called attention to a similar association (Wolf,⁶ Sehart,⁸ Perrone,³⁷ Friedlander³⁰). In thirty-one carcinomas of the lung studied by Wolf,⁶ thirteen showed tuberculous lesions. He stated that bronchial carcinomas were usually found in places subjected to the greatest mechanical injury, that is, at the bifurcation of bronchi of various orders, where on account of the angulation, irritation from dust or other inspired particles is greatest. His theory, often designated by the term "Pigmentdurchbruch," proposed that anthracotic tuberculous lymph nodes at these points rupture into the bronchi and thus acting as the source of a chronic irritant lead to cancer. He described such lesions, but his ideas and work have not received support and confirmation. Friedlander,³⁰ Perrone,³⁷ Wolf,⁶ Kaminsky,³⁸ Neumeister,³⁹ and Gougerot⁴⁰ have described squamous cell carcinoma, and Schwalbe⁴¹ a cylindrical cell carcinoma arising in the walls of tuberculous cavities.

CASE REPORTS

CASE 1—*Clinical History*—J. P., a white female, aged 67 years, was admitted to the New Haven Hospital, May 27, 1918, in an unconscious condition. The family history was irrelevant. The personal history revealed the fact that the patient had suffered from a cough and bronchitis since childhood. The

35 Menetrier, P. Cancer primitif du poutmon, *Prog. med.* **3** 436, 1886.

36 Ribbert, H. Bemerkungen zu einem Falle von primaren Lungencarcinom, *Deutsch. med. Wchnschr.* **22** 165, 1896.

37 Perrone, A. Entwicklung eines primaren Kankroids von der wand einer tuberculosen Lungenkaverne, *Arch. d. path. Inst. zu Berlin*, Feiler J. Orth 1906, p. 235.

38 Kaminsky, M. M. Ueber primaren Lungenkrebs, *Inaug. Diss.* Greifswald, 1898. (Quoted by Perrone³⁷.)

39 Neumeister, K. Ein Fall von primaren Plattenepithelkarzinom der lunge mit metastase im Schultergelenk, *Munchen med. Wchnschr.* **52** 1721, 1905. (Quoted by Perrone³⁷.)

40 Gougerot, H. Cancer primitif du poutmon a globes epidermique, *Bull. et mem. Soc. anat. de Par.* **80** 294, 1905. (Quoted by Perrone³⁷.)

41 Schwalbe, E. Entwicklung eines primaren Carcinoms in einer tuberculosen Caverne, *Virchows Arch. f. path. Anat.* **149** 329, 1897.



Fig 1—Case 1 Microscopic drawing of a peribronchial tumor nodule. The bronchial wall is thickened. The tumor forms solid nests of epithelial cells resembling the overlying metaplastic mucous membrane and numerous glandular alveoli suggesting the bronchial mucous glands.

present illness was completely dominated by symptoms of cerebral hemorrhage. The examination was confirmatory of this condition. The patient did not recover consciousness and died about nine hours after admission.

Necropsy 59 (Performed by Dr. McKinlay about twelve hours after death)

Anatomic Diagnosis—Carcinoma of the bronchus, multiple metastases to the lungs, heart muscle, hilar and retroperitoneal lymph nodes, suprarenals, and thyroid. *Subsidiary* Chronic mitral and aortic verrucose endocarditis, cardiac hypertrophy and dilatation, cholelithiasis, chronic cholecystitis and fibrous pericholecystitis, encapsulated tuberculous lymphadenitis.

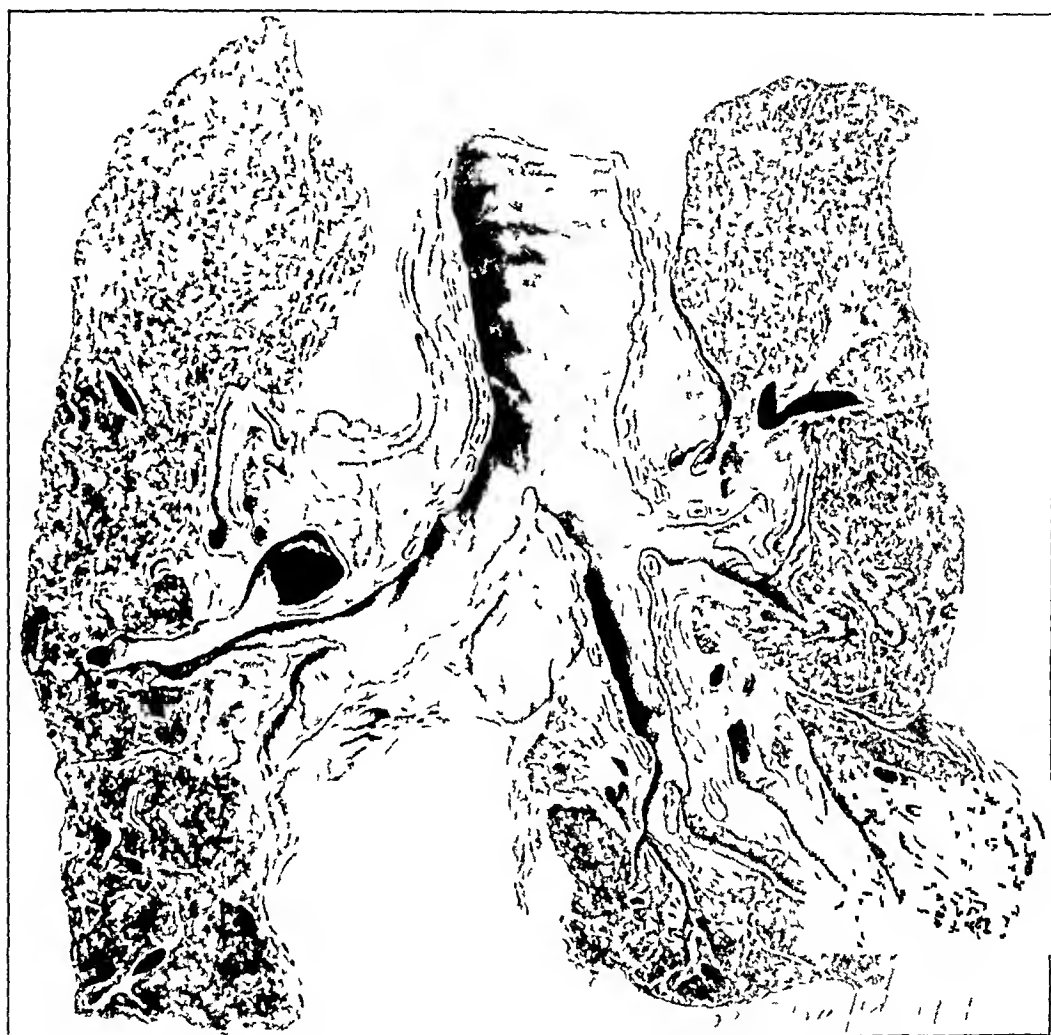


Fig. 2—Case 2. Primary carcinoma of the bronchus (posterior view). The primary tumor is shown projecting into the lumen of the bronchus to the right lower lobe just beyond a point of bifurcation. The tumor surrounds the larger bronchi in the right lung and fills the angle beneath the tracheal bifurcation. The hilar and peritracheal lymph nodes on both sides are involved but there are no tumor nodules in the left lung.

Protocol—(Abstract) The body is that of a fairly well nourished female, measuring 155 cm in length and weighing 112 pounds. Rigor mortis is present. There is marked postmortem lividity of the back. The pupils are equal and regular and the conjunctivae are pale. The thorax is flat. The abdomen is distended. The peritoneal surfaces are everywhere smooth and glistening and there is no excess of fluid. The liver extends 10 cm below the costal margin in the right midclavicular line. The left pleural cavity contains about 100 cc

of clear straw colored fluid. There are a few fibrous adhesions between the parietal and visceral pleurae over the lateral aspects of the upper lobe. The right pleural cavity contains about 300 c.c. of clear straw colored fluid. The pericardial sac presents nothing abnormal.

Lungs. There are small white, fibrous scars at both apices. There are also numerous fibrous tags on the pleural surfaces. The lungs crepitate throughout but have a nodular consistence with areas of emphysema at the periphery of the lobes. The cut surface of the lungs appears red in color, and is mottled with black pigmented areas. Throughout the lungs the blood vessels and bronchi are prominent on account of the surrounding grayish-white areas which vary from narrow rims to broad bands extending as much as a centi-

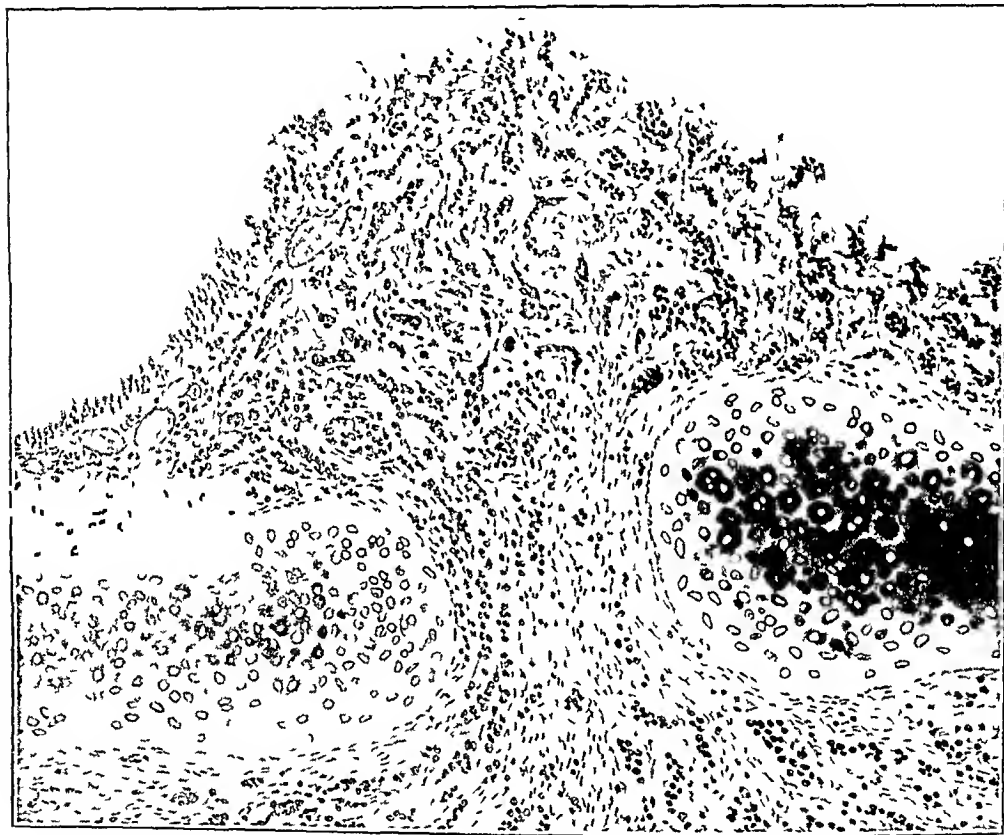


Fig 3—Case 2. Microscopic drawing of the primary bronchial tumor shown in Figure 2. The hyperplastic surface epithelium is seen on the left of the drawing. The nests of carcinoma cells extend along the free surface and throughout the bronchial wall. These cell nests resemble the proliferated bronchial epithelium seen in influenza (compare Fig 15).

meter into the surrounding tissue. These areas are firm in consistence and grayish-white in color. The condition is somewhat more marked in the right lung. The hilar lymph nodes are markedly enlarged, some measuring 3 cm. in diameter. The cut section appears grayish-white in color. Some are translucent and others are opaque in appearance and on pressure a white mucoid material exudes from the centers.

Metastases. There are tumor nodules in both suprarenal glands, the retroperitoneal, mediastinal and the hilar lymph nodes. These nodules resemble the tumor in the lung. The gross findings in the other organs have no relationship to the tumor and will not be described.

Microscopic Notes—Lungs The lungs present several interesting findings. The first is the tumor. There are numerous circumscribed carcinoma nodules situated chiefly in the peribronchial region (Fig 1). They are composed of a fibrous background within which numerous small, irregularly shaped, solid masses and strands of epithelial cells are seen. These cell nests extend through the submucosa, reaching up to, and almost encroaching on, the mucous membrane. The cells are large and polyhedral in shape with large nuclei. The nuclei vary greatly in size, shape, and chromatin content. They vary from the deeply pyknotic to clear vesicular nuclei with large central nucleoli. Mitotic figures are numerous. The cells resemble normal squamous epithelium but there are no epithelial whorls or areas of keratinization. In many areas the overlying bronchial mucous membrane is metaplastic and forms numerous layers of stratified epithelium. There is no definite basement membrane and there are areas suggesting a continuity between the tumor and the

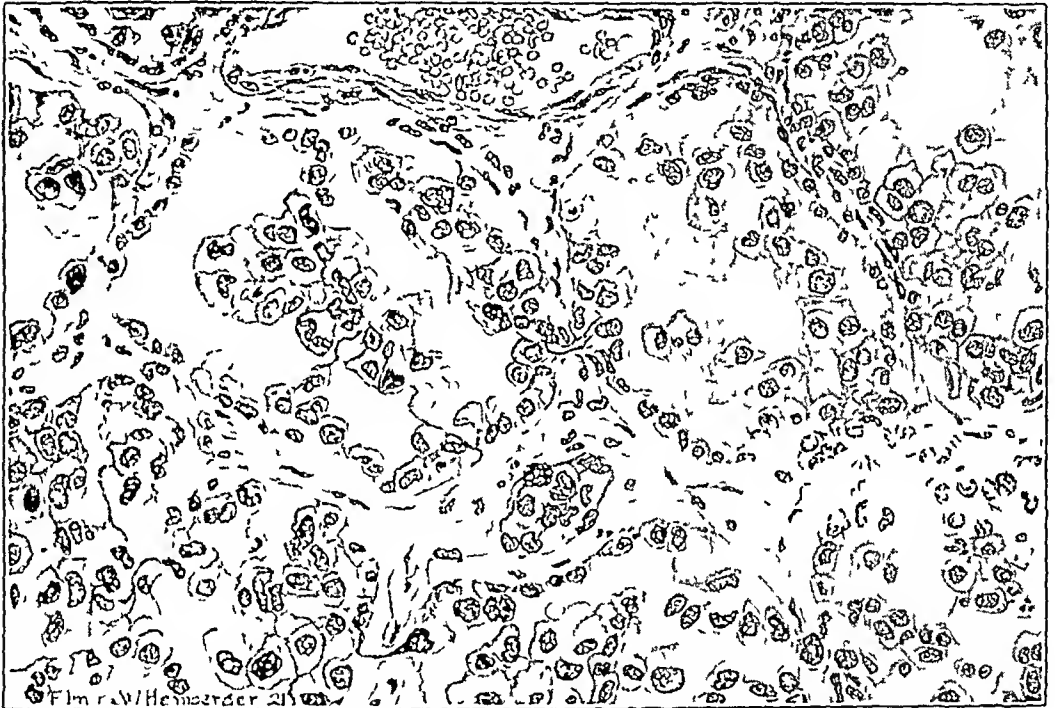


Fig 4—Case 2. Microscopic drawing showing area in which the tumor has invaded the air spaces. The masses of tumor in adjacent alveoli are connected through openings in the alveolar walls. The tumor cells fill the lumina or extend along walls of the alveoli.

metaplastic mucosa. The resemblance between the tumor cells and the cells of the overlying mucous membrane is striking. However, in other areas a different picture is seen. There are areas of carcinomatous alveoli of varying form and size. Some are lined with a single layer of columnar epithelium and a few are histologically indistinguishable from the normal bronchial mucous glands. Others are markedly irregular in size and shape, showing infoldings and outgrowths of the lining cells with all gradations to the solid masses. In some places mucous secretion is evident. The other findings of interest are evidences of a preceding organizing inflammatory process and the changes in the alveolar epithelium. There are numerous organized thrombi, areas with definite thickening of the alveolar walls and fibrotic areas in the perivascular and peribronchial regions in sections free from any tumor. There are also areas in which the alveolar epithelium is distinctly cuboidal and occasionally forms several layers. This is most conspicuous in the scarred areas.

Metastases ⁴² There are numerous cancer emboli in the vessels of the myocardium. The suprarenals and the thyroid show tumor nodules with a structure similar to that of the primary tumor in the lungs. In some areas the cells resemble those of metaplastic bronchial epithelium. The lymph nodes show a similar type of tumor, as well as areas in which the cells are arranged irregularly in a loose meshwork.

Summary—The history of bronchitis in early life and the presence, at necropsy, of widespread areas of organization in the lungs are evidences of the existence of a preceding organizing inflammatory process. There was marked variation in the histologic characteristics of this tumor with areas



Fig 5—Case 3. Carcinoma of the bronchus (gross drawing). The primary tumor is seen filling the first branch of the main bronchus to the left lung. There is extensive infiltration of the left lung. Tumor nodules are seen within the pericardium and the right auricle. There is an oval mass of tumor projecting into the trachea at the bifurcation and a large mass filling the mediastinum.

⁴² The history suggests cranial metastases, but this could not be determined as permission for an examination of the head was not obtained.

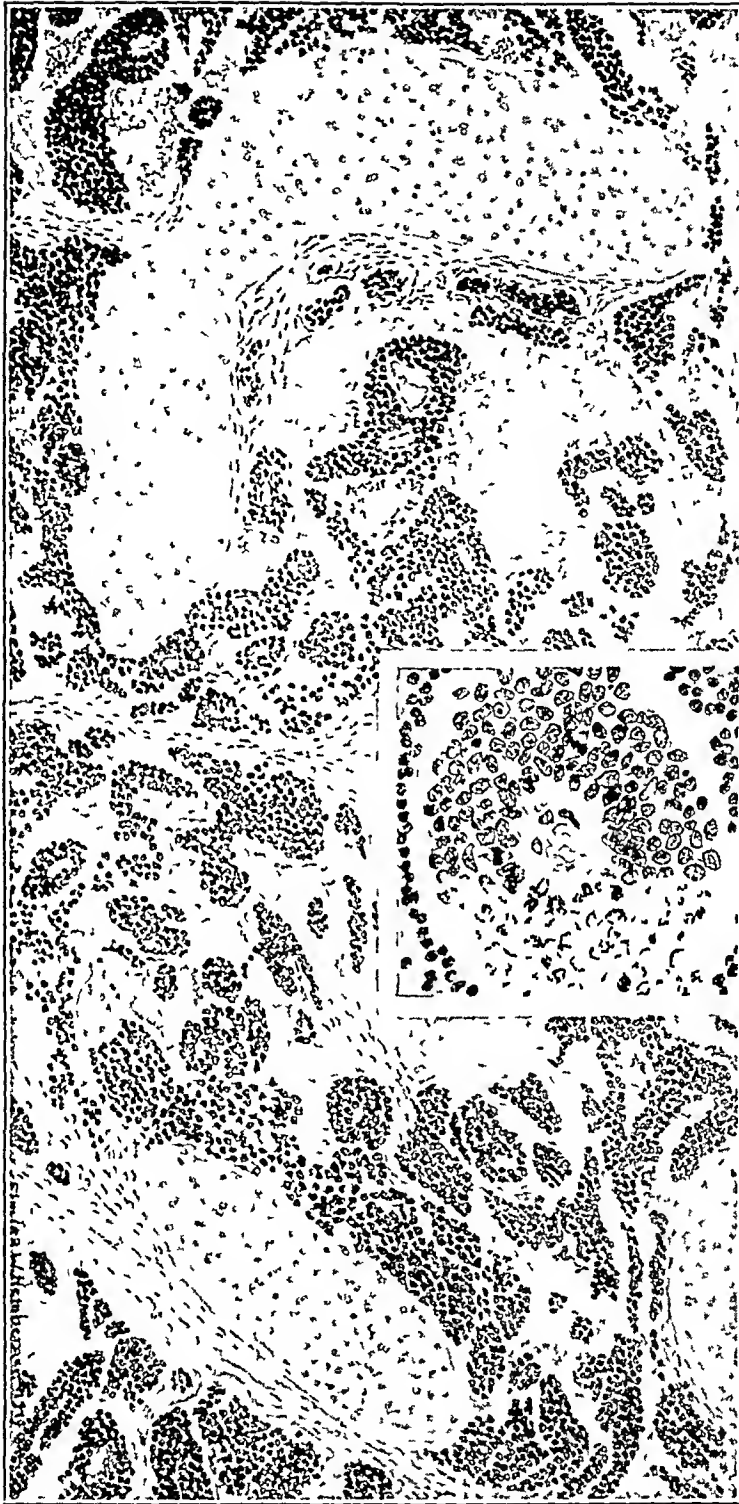


Fig 6—Case 3 Microscopic drawing of a section through plugged bronchus (primary tumor) showing islands of cancer cells which in many places surround small blood vessels There are extensive areas of necrosis and fibrosis This picture is uniform throughout the tumor

resembling the metaplastic bronchial mucous membrane, as well as areas suggesting the bronchial mucous glands. The metastases were of widespread distribution and resembled the peribronchial nodules. The exact location of the primary site was not determined and a question might be raised concerning the bronchial origin of this tumor. However, there was no evidence, grossly or microscopically, of a primary tumor in any other organ, although a careful search was made. The tumor nodules within the lungs were confined to the peribronchial area, a lymphatic distribution, which is in favor of a primary rather than a secondary tumor. While the bronchial origin cannot be conclusively proved a consideration of the case as a whole shows that the evidence is undoubtedly in favor of this diagnosis.

CASE 2—*Clinical History*—G K, a white male, aged 60 years, was admitted to the New Haven Hospital, Feb 22, 1919, complaining of breathlessness, cough



Fig 7—Case 4 Primary carcinoma of the bronchus (gross drawing) The external view shows a depressed scar on the surface of the left upper lobe. Small secondary tumor nodules are seen on the pleural surface. The cross section shows the primary tumor just beneath the depressed area on the surface. The tumor had its origin in a small bronchus just peripheral to the point of bifurcation.

and pain in the back. The family history and personal history are unimportant. The present illness began about three months before admission with pain in the back and girdle pains about the waist. During this time he has also suffered from gradually increasing dyspnea. For the last three weeks he has had a severe cough with considerable expectoration and has been unable to sleep well on account of orthopnea.

Physical Examination—The patient was considerably emaciated and was breathing with some difficulty. The cervical lymph nodes were slightly enlarged. There was a firm, bony, hard mass just above the left clavicle and a small nodule on the third rib just to the right of the sternum. The chest was emphysematous in type. In front the percussion note was hyperresonant except for slight impairment at the right apex. At the right apex and just below the right clavicle the sounds were bronchial, elsewhere the breath sounds were harsh. There was marked dullness and the breath sounds were distant over the entire right back. On the left, the percussion note was slightly hyperresonant and the respirations were harsh. The heart showed nothing of importance. The liver edge was palpable two fingers' breadth below the right costal margin.

The patient died suddenly on the evening of Feb 23, 1919.

Necropsy 204 (Performed by Dr McNamara about ten hours after death)

Anatomic Diagnosis—Carcinoma of the bronchus, invasion of the lung, metastases to the regional, cervical, retroperitoneal and mesenteric lymph nodes, suprarenals, pancreas and kidneys, invasion of cancer into the wall of the vena cava, ascites, occlusion of bronchus, local pulmonary atelectasis, hydrothorax, cancer thrombi in the pulmonary vessels, *propagated pulmonary thrombus* (stem of pulmonary artery). Subsidiary Apical pulmonary scars, hydrocele.

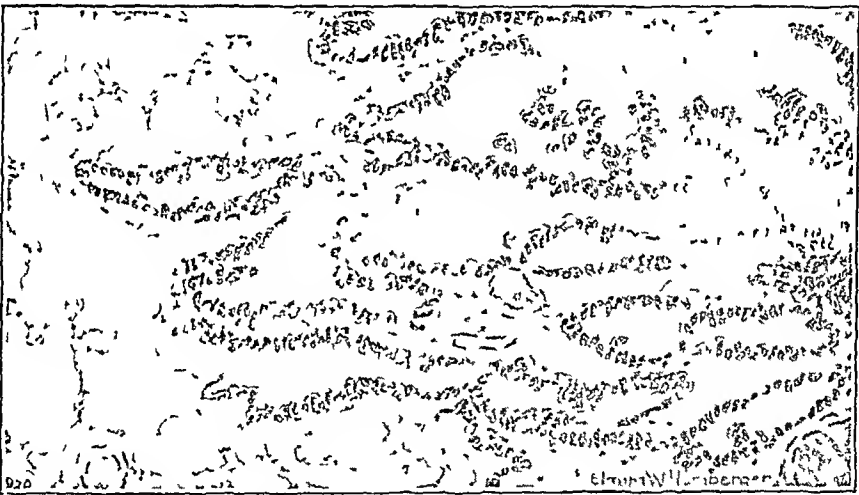


Fig 8—Case 4 Microscopic drawing of primary tumor of bronchus. The section is taken from the periphery of the primary tumor. There is a resemblance to regenerative alveolar epithelium and to tumors described as arising from the alveolar epithelium.

Protocol—(Abstract) The body is that of a poorly nourished white male weighing 156 pounds and measuring 170 cm in length. There is a firm mass just above the inner end of the left clavicle and a firm nodule adherent to the third rib just to the right of the sternum. On section this nodule is pearly gray and translucent in appearance and does not invade the rib. The abdominal wall, the back, the scrotum and the extremities are definitely edematous. The peritoneal surfaces are everywhere smooth and glistening and the cavity contains about 350 cc of clear, straw colored fluid. The edge of the liver extends 15 cm below the tip of the xiphoid and 11 cm below the costal margin in the right midclavicular line. There is a firm nodular tumor about the size of a lemon, in the region of the head of the pancreas. The mesenteric and retroperitoneal lymph nodes are enlarged.

The right pleural cavity contains about 2,000 cc of clear straw colored fluid and the lung is collapsed. The left pleural cavity contains no fluid. There

are a few fibrous adhesions at both apices. The anterior mediastinal tissues are edematous. The pericardial cavity contains about 100 c.c. of a blood tinged fluid. The serous surfaces are smooth and glistening.

Heart. The right side of the heart and the pulmonary artery are greatly distended while the ascending portion of the aorta is collapsed. After making an incision into the right ventricle and extending it into the pulmonary artery, a large thrombus is seen filling both branches of the pulmonary artery and extending back to the pulmonary valve. The remainder of the heart shows nothing abnormal.

Lungs. There are apical scars and a few fibrous adhesions in this region as well as over the lateral and posterior portions of the upper lobes of both lungs. The right lung is small in volume due to compression by the large pleural effusion. Externally, the lungs show no other abnormalities. On

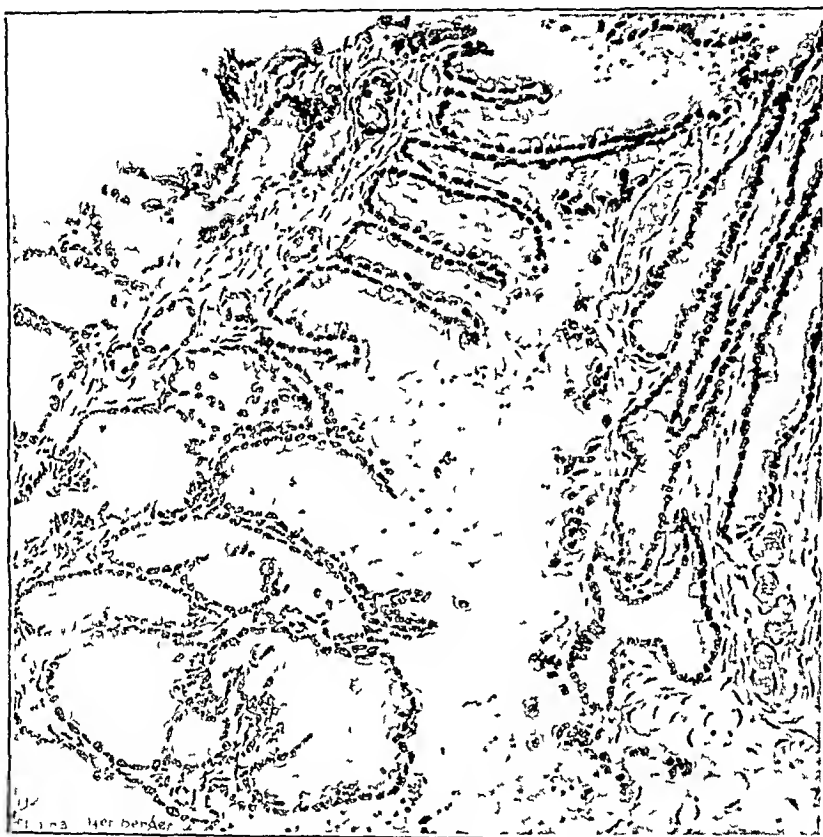


Fig 9—Case 4. Microscopic drawing of a section of the primary nodule. The carcinomatous alveoli resemble normal bronchial mucous glands with a tendency to formation of papillary ingrowths. Mucous secretion is marked. The picture conforms to the description of tumors arising in the bronchial mucous glands.

palpation there is a large, sharply localized, firm mass in the right lower lobe near the hilum. A frontal section, dividing the trachea, bronchi and lungs, reveals an interesting condition. A striking feature is the prominence of the thrombus which protrude from the arterioles over the entire surface. The tracheal mucosa is quite normal. In the main bronchi the mucosa is smooth, but intensely injected. Just below the bifurcation of the bronchus to the right lower lobe, there is an abrupt change. A firm mass with an irregularly raised surface is seen practically occluding the lumen for about 15 cm (Fig 2). Below this mass, there is a similar change back to the normal

mucosa The terminal portion of the bronchus is dilated This nodule is probably the primary site The configuration of the invasion of the lung is beautifully seen The peribronchial and perivascular regions are everywhere outlined by encircling bands of tumor which become more and more conspicuous as the hilum is approached This pathway leads to a large mass in the angle beneath the tracheal bifurcation, and can be traced up through the mediastinum to the left supraclavicular region The iliac glands on the left are also involved, but numerous sections show no metastases in the left lung

Metastases The right kidney and the suprarenals show tumor nodules There is a firm tumor mass, apparently composed of enlarged lymph nodes, near the head of the pancreas This mass also extends into the substance of



Fig 10—Case 4 Microscopic drawing of metastasis in ovary The resemblance to a small bronchiole is striking

the pancreas The cervical, iliac, mesenteric and retroperitoneal lymph nodes contain metastases The retroperitoneal lymph nodes compress the vena cava and its tributaries, and just above the junction of the iliac veins there is a tumor nodule projecting into the lumen of the vena cava There are also several areas of partial atrophy of the wall of the vena cava as the result of pressure by the carcinoma nodules These metastases resemble the primary tumor

Microscopic Notes—Lungs At the periphery of the primary tumor, the mass projects into the main bronchus to the left lower lobe, the bronchial mucosa varies from the normal to several layers of hyperplastic epithelium

The cells are somewhat irregularly arranged and are many layers deep, but vary only slightly from the usual cylindrical lining cells. The central portion shows complete loss of the mucous membrane with cancer cells extending along the free surface (Fig 3). The cells form irregular strands and solid nests invading the bronchial wall extensively and extending in either direction in the submucosa between the cartilages and into the peribronchial tissue. The cells are somewhat polymorphous and their arrangement suggests squamous epithelium. There are no epithelial pearls or areas of keratinization. The cells are large and the nuclei vary greatly in chromatin content. Mitotic figures are numerous. The resemblance of these cells to the bronchial epithelium is suggestive and immediately recalls the islands of proliferated bronchial epithelium observed in the recent influenza epidemic.



Fig 11—Case 4 Microscopic drawing of small bronchus with surrounding carcinoma. The carcinoma cells are seen pushing through the wall into the lumen of the bronchus.

In neighboring areas, however, the bronchial mucous glands are hyperplastic and there are changes present which suggest that they may be participating in the neoplastic growth. The comparative uniformity of the tumor elsewhere is against this suggestion despite the apparent hyperactivity of the glands, and the changes are probably only secondarily related to the tumor. In other sections the alveolar epithelium is distinctly cuboidal and in some alveoli is piled up to form several layers.

In the surrounding lung the alveoli are filled with solid nests of tumor cells. These cells likewise form syncytial masses and irregular alveoli. There are

many areas in which the carcinoma cells extend through the alveolar walls forming figures resembling closely the fibrin molds of the alveoli extending through the so-called pores of Cohn frequently seen in the pneumonic lung (Fig 4) The cancer cells also extend along the alveolar walls replacing the lining epithelium In some areas it is difficult to differentiate tumor cells growing along the alveolar walls from regenerating alveolar epithelium In addition to the tumor masses in the alveolar spaces there are peribronchial and perivascular nodules and tumor cells are seen within many of the blood vessels and lymphatics There are nodules extending to the pleura but not invading it The lymphatic channels of the interlobular septa contain many cancer cells Large polyhedral cells predominate but many cuboidal and flattened, pavement-like cells are present The nuclei are large and numerous mitotic figures are present The cytoplasm is finely granular In many places several nuclei are seen in an undifferentiated mass of cytoplasm

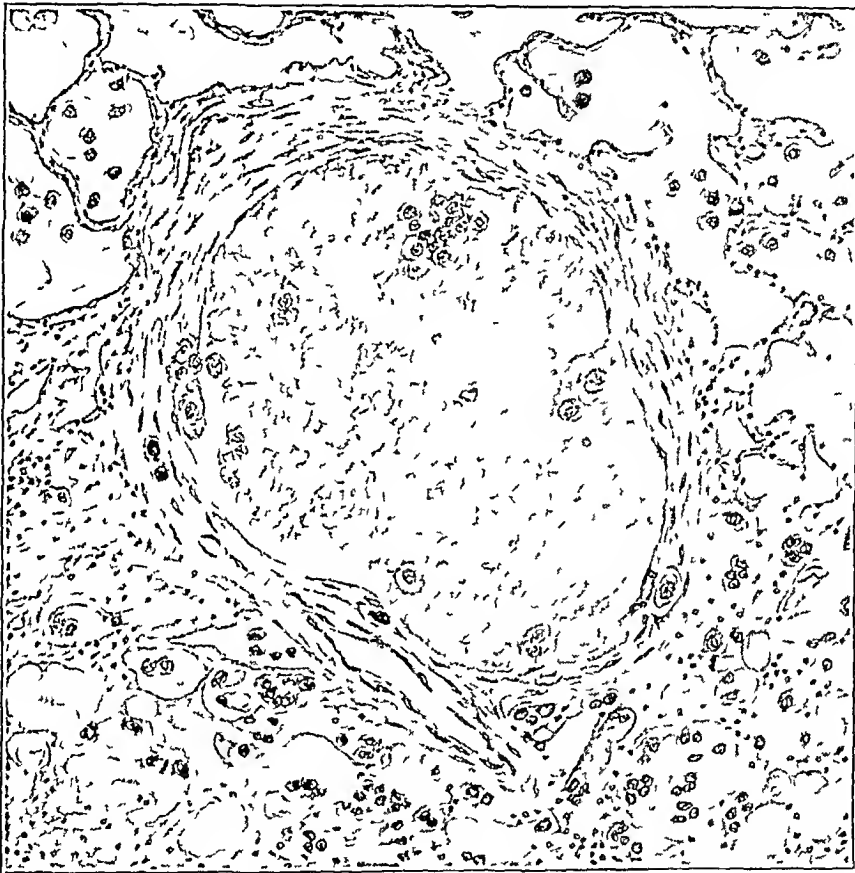


Fig 12—Case 4 The drawing shows carcinoma cells in the wall and within the lumen of a small pulmonary blood vessel

In all sections the blood vessels are conspicuously congested and in some there are fresh as well as organizing thrombi In some areas there is a hemorrhagic exudate in the alveolar spaces There are numerous areas of peribronchial and perivascular fibrosis There is no evidence of tumor in the left lung although the fresh and organizing thrombi as well as the fibrotic peribronchial and perivascular areas are present

Metastases The suprarenals, the right kidney and the cervical, retroperitoneal and mesenteric lymph nodes show metastases resembling the tumor in the lung

Summary—There were widespread areas of peribronchial and perivascular organization as evidence of a preceding organizing inflammatory process The

tumor was largely situated in the peribronchial and perivascular regions, but there was also invasion of the pulmonary parenchyma

The majority of the tumor cells formed strands and solid nests of polymorphous cells but there were some areas showing a glandular structure. The metastases were of widespread distribution and resembled the primary tumor. The tumor was undoubtedly of primary bronchial origin, and probably began in the nodule projecting into the main bronchus to the right lower lobe.

CASE 3—*Clinical History*—I M., a white male, aged 39 years, was admitted to the New Haven Hospital, Oct 4, 1919, complaining of swelling of the left

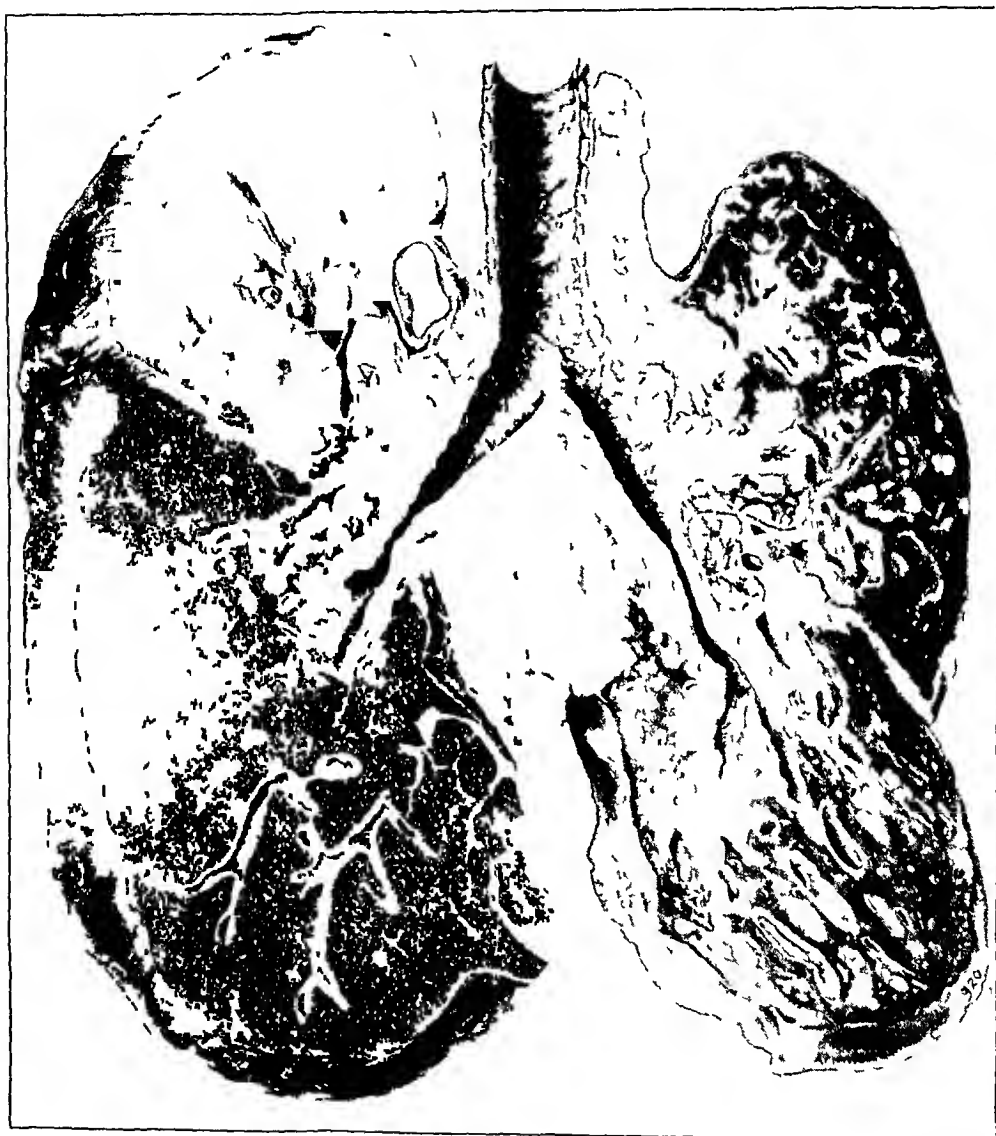


Fig 13—Case 5 Primary carcinoma of the bronchus (posterior view). The tumor involves the lower portion of the trachea and both main bronchi with widespread invasion of the right lung and bronchial lymph nodes. The probable point of origin, in first bronchus to the right upper lobe does not appear in this section (see text).

arm. The history is meager as the patient spoke very little English. The patient has had cough with expectoration for five years and has had slight hemoptysis during the past year. He has had no night sweats. About Sept

20, 1919, he began to suffer from pain in the right upper part of the chest radiating over the left shoulder and down the arm. This arm has gradually become swollen and the pain has decreased.

Physical Examination—The physical examination showed evidence of an intrathoracic tumor. There was no precordial pulsation or tracheal tug. There were enlarged axillary lymph nodes on the left side with swelling of the shoulder and arm and evidence of a well developed collateral circulation in this region. There was a myosis of the left pupil, ptosis of the left eyelid, and paralysis of the left vocal cord. There was impairment of the percussion note over the entire left chest with bronchial breathing over a wide area.

Clinical Diagnosis—Neoplasm of left upper lobe of lung, probably carcinoma.

The patient manifested difficulty in breathing which gradually became more marked until death on Nov 11, 1919.

Necropsy 269 (Performed by Dr Alter about four hours after death)—

Anatomic Diagnosis—Carcinoma of the bronchus, invasion of the left lung, mediastinum, pericardium and left auricle, occlusion of bronchus, chronic



Fig 14—Case 5 Microscopic drawing of tumor in left lung. The tumor is a typical keratinizing squamous celled carcinoma.

tubular bronchiectasis, organized lobar pneumonia, fibrous pleurisy, compression of the aorta, organized thrombi in the axillary veins, edema of arm, emaciation.

Protocol—(Abstract) The body is that of an emaciated white male, measuring 162 cm in length and weighing 105 pounds. Postmortem lividity and rigor mortis are absent. The trachea shows deviation to the left side and is firmly fixed. The supraclavicular glands are enlarged and firm. There is nothing of note in the abdominal cavity. The mediastinum is filled by a large, yellowish-white mass which is firmly adherent to the anterior thoracic wall and seems to extend up from the left lung. The left pleural cavity is completely obliterated. There are extensive adhesions between the parietal and visceral pleurae on both sides. The heart is pushed downward and to the left. After removing the lungs with the neck organs by dissecting the parietal pleura from the chest wall, only a relatively small amount of tumor remains attached to the bony structures surrounding the thoracic aperture. The trachea is virtually embedded in the tumor mass. The axillary veins form solid cords and on section are filled with organizing thrombi.

Lungs The mediastinal organs are embedded in the large mass which measures about 13 cm in its greatest diameter (Fig 5) A transverse section through the left lung shows the distribution of the neoplasm It extends upward along the trachea, surrounding and compressing the aortic arch and filling the mediastinal space Downward it extends along the bronchi and



Fig 15—(Necropsy 183) Microscopic drawing of the proliferative changes in the bronchial epithelium in influenza There is a partial obliterative bronchiolitis and organization of the surrounding lung tissue The bronchial epithelium is several layers thick and has invaded the surrounding organizing tissue, giving the picture of an early carcinoma

the larger vessels, into the lung along the ramifications of the vessels and bronchioles which stand out as thick walled tubes It likewise extends along the larger vessels into the pericardial sac forming a polypoid mass around the pulmonary vessels It invades the heart wall and projects into the cavity of

the left auricle as small, knoblike growths. The tumor projects into the bronchi in many places, apparently by extension from adjacent tumor nodules. The bronchial walls are thick, they are lined by a red, inflamed mucous membrane and contain a large amount of purulent material. In the upper and middle portions of the lung, there are numerous tumor nodules varying greatly in size. Just beyond the bifurcation of the main bronchus to the left lung, there is a mass, apparently the oldest portion of the tumor, surrounding, filling and completely occluding the lumen. This is probably the primary site. There is an elongated, oval mass, 5 cm in length, projecting into the lumen of the trachea for about 1 cm. It extends up from the subjacent tumor mass and its surface is smooth and covered with epithelium. Many of the larger bronchi are somewhat compressed by the surrounding tumor. On section the larger masses resemble a rapidly growing medullary carcinoma with many areas of necrosis. The smaller masses are grayish-white in color and translucent in appearance.

The lower lobe is the site of an extensive inflammatory process. Only narrow areas are air-containing. The bronchi are somewhat compressed, but not obstructed, and can be followed to the periphery where they end in cylindrical dilatations. They are filled with necrotic brown debris.

The right lung is uninvolved and there are no metastases outside of the thoracic cavity.

Microscopic Notes—Lungs. The sections through the primary site show some distortion of the cartilages by the surrounding growth (Fig 6). The lumen is obliterated by the neoplasm. The tumor cells are best preserved around the blood vessels where they form round or irregular concentric nests surrounded by areas of necrosis or fibrosis. Radiating from the central vessel, there is an inner row of cylindrical cells surrounded by other cells irregularly arranged and closely packed together so that the shape of individual cells is less definitely discernible. The nuclei are relatively large and show many mitotic figures. There is no intercellular stroma.

With minor variations, this structure prevails throughout the tumor. There are a few areas in which long strands of cylindrical cells are present and many areas showing large masses of irregularly arranged cells. In the lung proper, there are areas in which the alveolar spaces contain masses of tumor cells. These areas are poorly preserved. In some places tumor cells extend through the alveolar walls connecting the tumor masses in neighboring alveoli, as was described in Case 2.

Sections from the lower lobe show an extensive organizing process with numerous bronchiectatic dilatations. There are areas of thickening of the alveolar walls with all gradations to completely fibrosed areas. The alveolar spaces are totally or partially obliterated and the lining epithelium is composed of a single layer or several layers of cuboidal cells. There is organization in areas distant from tumor nodules and portions of the lungs reached by compressed bronchi.

Microscopic examination of the other organs shows nothing of importance in connection with the tumor.

Summary—The extensive organization was undoubtedly due to compression of the bronchi. However, there were changes, areas of organization distant from portions of the lung reached by compressed bronchi, indistinguishable from those seen in late cases of influenza, and there is little, if any, doubt concerning the existence of a preceding organizing inflammatory process. The history also confirmed this view. The distribution of the tumor was entirely within the thoracic cavity. The right lung was uninvolved. The left lung showed a tumor of peribronchial and perivascular distribution with many areas of invasion of the pulmonary parenchyma. The histological picture was uniform throughout, showing high cylindrical cells frequently arranged in irregular perivascular rosettes. There is no doubt concerning the bronchial

origin of this tumor, and there is presumptive evidence that the site of the origin was in the first left bronchus

CASE 4—Clinical History—G B, a white female, 42 years old, was admitted to the New Haven Hospital, Oct 10, 1919, complaining of pain in the right knee and leg, cough, soreness in her chest, and blindness in the left eye. The family history shows that her father died of cancer of the liver, a paternal aunt of cancer of the breast, and her paternal grandfather of cancer of the stomach. The patient's sister had a kidney removed for a growth, the nature of which is not known. The personal history reveals an attack of dry pleurisy at the age of 16 years, a severe cough lasting several months at the age of 25 years, and a definite attack of influenza in March, 1919.

The present illness began in June, 1919, with a cough. At the same time she had pain in her right thigh and knee and some weakness in her legs. In August she developed diplopia, followed a week later by complete blindness in the left eye. In September she began to suffer from dyspnea and severe paroxysms of coughing associated with vomiting and occasionally with blood tinged sputum. There have been no night sweats. While in the hospital she became blind in the right eye.

Physical Examination—The physical examination showed evidence of a malignant tumor with metastases in the left lung, the liver, the cranium, the ribs, and a generalized involvement of the lymph nodes. A lymph node was removed for diagnosis and showed an adenocarcinoma. On account of a mass felt in the region of the right kidney a clinical diagnosis of adenocarcinoma, probably primary in the right kidney, was made.

Necropsy 272 (Performed by me about three and one-half hours after death)
—**Anatomic Diagnosis**—Carcinoma of the bronchus with invasion of the lung, generalized metastases, including lymph nodes, bones, adrenal, ovary and liver, cancer emboli in the pulmonary artery, infarcts of lung, emaciation.

Protocol—(Abstract) The body is that of a markedly emaciated white female, measuring 172 cm in length and weighing 95 pounds. Rigor mortis is not present. There is slight postmortem lividity of the back. There is a definite protrusion of the left eyeball. The pupils are equal and regular, measuring 5 mm in diameter. There is a palpable mass in the right supraclavicular region and a small nodule in the left inguinal region. The peritoneal surfaces are everywhere smooth and glistening. The liver edge extends 65 cm below the xiphoid process and 4 cm below the costal margin in the right midclavicular line. There are numerous nodules along the edge of the liver. The pleural surfaces are everywhere smooth and glistening. There are several small, hard nodules firmly adherent to the ribs on the right side.

Lungs Externally there is a sharp line of demarcation between the lower portion of the left upper lobe, which is firm in consistence and dark reddish purple in color and the remaining lighter colored, normal appearing lung (Fig 7). Near the edge of this area, bordering on the interlobar fissure, there is a horizontal scar about 4 cm in length. The scar is puckered and the center is depressed. On the surface there are numerous small, pea sized, elevated nodules. A section through the center of the scar shows a small mass about 1.5 cm in diameter which is in all probability the primary nodule. It is firm in consistence, grayish-yellow in color and of an irregular rosette shape. A small bronchus runs into the mass just after bifurcating. The surrounding lung tissue is firm and dark except for numerous white bands outlining the interlobular spaces and surrounding the bronchioles. The darker areas have a homogeneous smooth, waxy appearance. There is a sharp line of demarcation similar to that described on the surface. The remainder of the lung shows a widespread infiltration of the tumor. The distribution is chiefly in the peribronchial and perivascular areas where grayish-white bands are seen forming collars around the bronchi and vessels which become progressively larger as the hilum is approached. In several places the growth has invaded the bronchi, forming nodular projections into the lumina with or without

ulceration of the mucous membrane. The right lung is normal in appearance, except for two small infarcts at the lower margin of the upper and lower lobes respectively. The hilar glands on both sides are enlarged and lead to a large mass filling the angle beneath the bifurcation of the trachea.

Metastases. The liver contains numerous tumor nodules scattered over the surface and throughout the substance. The suprarenals and ovaries also contain small white tumor masses. There is a chain of carcinomatous lymph nodes involving both sides of the neck and extending through the mediastinum into the retroperitoneal and pelvic nodes. The mesenteric, inguinal, and axillary nodes also are involved. On section these metastases appear as firm, white, translucent nodules resembling the tumor in the lung. There are also several firm nodules on the ribs and on the inner table of the cranial vault, as well as a diffuse infiltration of the floor of the anterior fossa of the base of the skull with extension along the optic nerve into the retrobulbar space.

Microscopic Notes—Lungs. The sections show a small bronchus extending into the proximal side of the primary nodule. The tumor fills the lumen of the bronchus and grows out peripherally spreading into a fan-shaped mass. The cartilaginous rings are somewhat distorted. In the proximal portion of the section the tumor is composed of numerous irregularly arranged cells with wide areas of necrosis. The central portion of the nodule shows a large amount of fibrous tissue with small alveoli or islands of epithelial cells. In the peripheral portion of the nodule, the following pictures are seen: (1) There are areas showing large and small alveoli lined with a single layer or many layers of cylindrical cells showing infoldings and papillary projections into their lumina. There are all gradations from simple alveoli lined with a single layer of cuboidal epithelium, to complicated intercommunicating alveoli with numerous infoldings and branching structures projecting into the lumina (Fig 8). (2) There are areas best described by saying that they resemble closely the bronchial mucous glands showing all of the gradations from an alveolus resembling the normal gland, to complicated branching forms covered with mucous secreting cells (Fig 9). Furthermore, the metastases, particularly in the lungs, also show areas resembling the bronchial mucous glands. (3) There are other areas showing polymorphous spaces lined with high cylindrical cells resembling the bronchial lining epithelium. This is even more striking in the metastases, particularly in the ovary where the resemblance to the smaller bronchi is vivid (Fig 10).

One section shows a bronchiole surrounded by many irregular alveoli and nests of cancer cells which have invaded the submucosa and the mucosa, and are seen pushing into the lumen as irregular shaped papillae and alveoli (Fig 11). In many sections of the lung, cancer cells are seen within the lumina and the walls of blood vessels (Fig 12). Many alveoli are filled with large polymorphous cells with large nuclei, and in some places many nuclei are seen within an irregular shaped mass of undifferentiated protoplasm. In some areas the cancer cells, in adjacent alveoli, are connected by extension through the alveolar walls as was described in Cases 2 and 3. In other areas the cancer cells extend along the alveolar walls, appearing as large polyhedral, cuboidal or flattened pavement-like cells replacing the alveolar epithelium. There are mitotic figures throughout the tumor. There are also many areas of necrosis. There are many widespread areas of peribronchial and perivascular organization distant from any tumor masses. There are small cancer emboli in the vessels leading to the infarcts in the right lung. Otherwise the right lung is conspicuously free from tumor.

Metastases. There are visceral metastases in the liver, suprarenals and lymph nodes. They resemble the primary tumor in structure. In some areas there is marked mucous secretion. The ovarian metastases have a striking resemblance to small bronchioles. There are bone metastases in the ribs and the skull. The tumor in the anterior fossa of the base of the skull extends into the orbits and compresses and invades the optic nerves. The structure is similar to that described elsewhere.

Summary—The interesting points in the history were the striking family history of malignancy and the attack of influenza three months prior to the onset of symptoms. There were many areas of peribronchial and perivascular organization as further evidence of a preceding organizing inflammatory process. In the left lung there was a widespread invasion of the peribronchial and perivascular regions with many areas of extension into the surrounding pulmonary parenchyma. In the right lung the metastases were widespread and resembled the primary tumor. Histologically, there were great variations in the findings, and areas were seen within the primary nodule fitting perfectly the authoritative descriptions of carcinomas arising from the bronchial mucous membrane, the bronchial mucous glands, and the alveolar epithelium. The primary nodule was situated beneath the scar in the left upper lobe. It was just beyond the bifurcation of a small bronchus, in a position from which a bronchial or an alveolar tumor might arise.

CASE 5—Clinical History—F. G., a white male, aged 45 years, was admitted to the New Haven Hospital, March 2, 1920, complaining of a cold and pain in his right side. The family and personal history are unimportant. The present illness began Feb. 1, 1920, with sudden sharp pain on the right side of his chest following severe exertion. At this time he developed a cough with profuse expectoration of blood stained mucus. The pain in the chest and the cough have persisted. He has also had fever, night sweats and increasing dyspnea on exertion. He has lost 45 pounds in weight during the last month.

Physical Examination—The physical examination on admission showed a markedly emaciated man. There were enlarged lymph nodes above the right clavicle. The right side of the thorax was somewhat flattened. At the right apex and down to the third rib in front, the percussion note was impaired and the expiratory sounds were prolonged. The signs did not suggest an active lesion at this time. The abdominal examination was negative. Ten days later the flattening of the right side had markedly increased, there was a conspicuous limitation of motion on this side and the supraclavicular lymph nodes had increased in size. The changes in the percussion note and the breath sounds were more pronounced, in front the retromammary dulness had distinctly increased toward the right, behind, there was dulness extending to the fourth dorsal spine, as well as over the entire base below the eighth dorsal spine. There was a slight kyphosis at the eighth and ninth dorsal vertebrae. A cervical lymph node was removed for diagnosis and the sections showed a metastatic carcinoma. A right sided pleural effusion developed later. Examinations of the sputum for tubercle bacilli were repeatedly negative. The pulmonary signs steadily increased, the fever, between 100 and 103 F., was continuous and there was increasing dyspnea and emaciation until death on June 28, 1920.

Clinical Diagnosis—Primary carcinoma, probably arising in a bronchus with invasion of the lung and mediastinum and metastases to the cervical lymph nodes and spinal column.

Necropsy 375 (Performed by Dr. Robinson about three days after death)—*Anatomic Diagnosis*—Carcinoma of the bronchus, invasion of the lung, pleura, mediastinum and pericardium, compression of the bronchi, chronic tubular bronchiectasis, metastases to the regional lymph nodes, liver, vertebrae, sternum and clavicle, fibrous pleurisy and pericarditis, emaciation, decubitus.

Protocol—(Abstract) The body is that of an emaciated white male, measuring 172 cm. in length and weighing 112 pounds. Rigor mortis is not present. The cervical lymph nodes are enlarged. The right side of the thorax is definitely flattened. There are decubitus ulcers over the fifth dorsal spine and the tip of the sacrum. The peritoneal surfaces are everywhere smooth and glistening and the abdominal organs are normally disposed. The liver edge extends 6 cm. below the costal margin in the right midclavicular line and there are numerous elevated nodules on the surface. There is a tumor mass.

filling the anterior mediastinum. The right pleural cavity is obliterated by extensive pleural adhesions. The left lung is entirely free from adhesions. The pericardial cavity is also obliterated by adhesions.

Lungs. After removal of the lungs, the mediastinal structures and the trachea en masse, a view of the tumor is obtained. There is a large yellowish-gray tumor filling the anterior mediastinum, surrounding the trachea, and continuing up to the lower border of the thyroid. There are extensive pleural adhesions over the entire right lung and there are numerous tumor nodules on the pleural surface. Numerous frontal sections through both lungs, the main bronchi, and the trachea reveal the distribution of the tumor. There is a large mass beneath the bifurcation of the trachea involving the hilic lymph nodes on both sides and compressing the main bronchi. There is also a large mass extending along the right side of the trachea with a smaller mass on the left. The parenchyma of the left lung shows no metastases. The right lung is extensively invaded by the tumor. The main bronchus to the right upper lobe communicates with a large cavity measuring about 5 cm in diameter. The walls of the cavity are necrotic and the lumen is filled with a thick mass of necrotic cellular debris. There is a large mass, apparently the oldest portion of the tumor, completely surrounding this cavity, measuring about 5 cm in its greatest diameter. This mass forms a wide band extending to the hilus and connecting with the mediastinal tumor.

In the less involved sections of the lung the usual picture is seen. There are bands of tumor encircling the bronchi and blood vessels (Fig 13). These perivascular and peribronchial tumor masses become progressively larger as the hilum is approached. There are no definite nodules projecting into the lumina of the bronchi, although the mucous membrane of the lower portion of the trachea and both main bronchi is absent and the surface is rough and finely granular in appearance. The bronchioles of the lower lobe are dilated and contain a creamy purulent exudate. A section of the tumor is yellowish-gray in color and the extensive areas of necrosis give the surface a lobular appearance.

Microscopic Notes—Lung. The sections of the right lung show a typical keratinizing squamous celled carcinoma (Fig 14). There are numerous strands and solid nests of large polyhedral cells infiltrating the peribronchial area. These cell nests are irregular in size and shape. Some are apparently situated in the lymphatic vessels, others are surrounded by a dense fibrous stroma. In many areas epithelial pearls and areas of keratinization are seen. There are areas in which carcinoma cells are seen filling the alveoli and in a few places the tumor cells in adjacent alveoli are connected by extension through the alveolar walls. Mitotic figures are numerous. Carcinoma cells are also seen within the blood vessels.

The bronchial mucous membrane is absent in many cases. In some bronchi the normal mucosa is present and in other areas the epithelium is hyperplastic and forms many layers of somewhat irregularly arranged cells. There are widespread areas of necrosis throughout the lung. In these areas the tumor cells and normal structures of the lung appear in phantom outline only.

The left lung shows only an occasional small accumulation of cancer cells in the peribronchial area. There is a widespread thickening of the alveolar walls.

Metastases. There are gross metastatic nodules in the hilic lymph nodes and in the liver and microscopic metastases in the pericardium. In the lymph nodes there are many epithelial pearls and areas of keratinization. In the liver there are nodules of densely packed, large polyhedral cells without areas of keratinization. Mitotic figures are conspicuous.

Summary—The widespread fibrous thickening of the alveolar walls and the metaplastic bronchial mucous membrane are evidences of a preceding inflammatory process. The right lung was extensively involved with widespread invasion of the peribronchial regions, as well as of the surrounding pulmonary parenchyma. There were only a few microscopic carcinomatous

nodules in the left lung Presumably the primary site was in the region of the cavity in the right upper lobe Histologically, the tumor was a typical squamous cell keratinizing carcinoma The metastases resembled the primary tumor There was no doubt concerning the primary bronchial character of this tumor, and it very probably arose in metaplastic bronchial mucous membrane

DISCUSSION

Incidence—The five cases reported in this paper have occurred in a consecutive series of 375 postmortem examinations among which there have been a total of twenty-nine carcinoma cases Although the series is small, these figures, 1.38 per cent of all necropsies and 17 per cent of all carcinomas, are high in comparison with those usually given

It may be noted, as regards sex, that three of the tumors were in males and two in females

Clinical Characteristics—The clinical characteristics may conveniently be discussed according to the four clinical groups outlined above, that is, (1) small solitary tumors producing no clinical symptoms, (2) tumors in which the dominant symptoms are produced by cerebral or other metastases, (3) those in which the patient is first seen in a moribund condition, and (4) those showing symptoms and signs of more or less extensive pulmonary involvement with or without metastases No case of this series belongs to the first comparatively rare group In group 2 there are Cases 1 and 4 In Case 1 the patient was unconscious when admitted to the hospital, and the history and physical examination were entirely dominated by cerebral symptoms This case may also be placed in group 3 In Case 4 there were symptoms and signs of a malignant tumor with generalized metastases involving particularly the brain and the lungs An excised lymph node showed an adenocarcinoma A mass was palpated in the region of the right kidney and a clinical diagnosis of an adenocarcinoma arising in this region was made In group 4, Cases 2, 3, and 5 are included In Case 2 there was evidence of extensive pulmonary involvement, enlarged cervical lymph nodes, and a nodule on the third rib, however, the sudden death of the patient from pulmonary thrombosis on the day after admission prevented a thorough clinical study In Case 3, there was evidence of an intrathoracic tumor with symptoms of pressure on the recurrent laryngeal nerve The clinical diagnosis was an intrathoracic carcinoma In Case 5, a clinical diagnosis of primary carcinoma of the bronchus was made This diagnosis was based upon the increasing right sided intrathoracic signs during the patient's stay in the hospital with a simultaneous enlargement of the cervical lymph nodes, one of which was excised and showed a metastatic carcinoma

In an analysis of the clinical aspects of bronchial tumors, Weller¹² emphasizes the value of bronchoscopic and radiographic examinations and the absence of fever and night sweats as important factors in

reaching a diagnosis. Bronchoscopic examinations of the five cases reported here were not made. It is probable that this method would have revealed the nodule in the lower portion of the trachea in Case 4 and the roughened and granular appearance of the lower portion of the trachea in Case 5. Such findings, however, would not have materially altered the diagnoses. Radiographic examinations showed the large mediastinal tumor in Case 4 and the progressive character of the pulmonary lesion in Case 5. Contrary to Weller's findings, however, fever was present in all five cases (the temperature ranging from 96 to 103 F), and night sweats were present in one case.

In three of the five cases a clinical diagnosis of a malignant tumor was made. In two of these the diagnosis was established by examinations of excised lymph nodes. In the other two cases a diagnosis was not possible since there was no opportunity for proper study.

Metastases—According to the extent of the metastases, carcinomas of the lung fall into three groups, (1) cases without metastases, (2) those with intrathoracic metastases only, and (3) those with generalized metastases. The cases reported in this paper fall into groups 2 and 3. Case 3 showed no metastases outside of the thoracic cavity. The remaining four cases showed widespread metastases.

As was stated above, a high percentage of lung carcinomas show generalized metastases. This may be explained by the close relation between lung tumors and the pulmonary circulation whereby tumor cells may be readily transported to the heart and into the general circulation. In favor of this suggestion the five cases reported here showed carcinoma cells within the pulmonary vessels, and, in Case 4, carcinoma cells are seen not only in the lumen, but also within the wall of a small vessel (Fig 12).

Furthermore, all five cases show evidence of invasion and spread through the lymphatic channels, and four show evidence of extension through the air spaces. The extension of lung tumors through the air spaces is important as areas are seen not infrequently in which it is difficult to differentiate between tumor cells growing along the alveolar walls and regenerative changes in the alveolar epithelium. Areas showing such alveolar extension may be confusing and lead to an incorrect diagnosis of alveolar origin.

Four of the cases afforded the interesting and unique observation of cancer cells extending through the alveolar walls. In some areas (Fig 4) this was quite striking and recalled the pictures of the fibrin bundles extending through the so-called pores of Cohn in a pneumonic lung.

Gross and Histologic Features—*Gross Features* The cases reported in this paper are rather advanced stages and show infiltrating tumors with very extensive involvement of one lung and the regional lymph

nodes Four cases present the picture that is most frequently described in primary bronchial carcinomas The only exception is Case 1, in which there were diffusely scattered peribronchial and perivascular tumor nodules, a picture possibly identical with the so-called miliary carcinosis of the older writers

Histologic Features The histologic findings in these cases are as follows (1) cylindrical cells (Cases 3 and 4) Case 4 is a typical cylindrical cell tumor showing a marked variation in the picture presented, and Case 3 is a cylindrical cell tumor showing a uniform picture, with a perivascular arrangement of the cells, (2) polyhedral cells (Cases 1 and 2) These cases show a predominant polyhedral cell with areas in which the arrangement and form of the cells suggest the squamous variety However, the absence of epithelial pearls, areas of keratinization or prickly cells is against this interpretation, and for the sake of exactness it seems advisable not to place tumors in the squamous cell group in which these findings are entirely absent, (3) squamous cells, Case 5 is a typical squamous cell tumor with epithelial pearls and areas of keratinization

Histogenesis—The question of histogenesis has absorbed the attention of many authors and it is unusual to find cases recorded in which a positive conclusion concerning the origin from the bronchial mucosa, the bronchial mucous glands, or the alveolar epithelium is not definitely stated However, the criteria on which the exact site of origin is determined have been variously stated by different authors and this, in itself, is an indication of the difficulties of reaching a correct conclusion There are a few cases of solitary papillomata arising in the wall of a bronchus or a tuberculous cavity in which the histogenesis is clear There are other cases generally accepted as of undoubted alveolar and bronchial mucous gland origin, notably those of Langhans⁴ and Kretschmar,³³ in which the diagnoses are probably correct However, the great majority of cases are seen in an advanced stage and it is difficult or impossible to determine the histogenesis accurately The points on which the diagnoses are made are not pathognomonic of alveolar and bronchial mucous gland tumors The chief criterion on which conclusions concerning the histogenesis of lung tumors have been made is resemblance between the tumor and the normal structures These resemblances are frequently striking and almost convincing, but the study of the present series of tumors shows, in one case (Case 3) at least, in a single tumor, areas resembling the authoritative descriptions of tumors arising from the bronchial lining epithelium, the bronchial mucous glands, and the alveolar epithelium These findings are seen within the primary tumor which is situated near the bifurcation of a small peripheral bronchus, a site from which any type of tumor might arise

It is also frequently stated that tumors arising in the bronchial mucous glands are largely situated in the bronchial wall producing a thickening of the wall and narrowing the lumen. But there is a serious fallacy in attributing much importance to this finding as one of the main lymphatic channels of the lung is situated in the bronchial wall and in the peribronchial area which constitutes, therefore, a natural line of growth for any malignant tumor of bronchial origin.

The bronchial lining epithelium and the bronchial mucous glands normally have mucus secreting cells and the presence of mucous secretion is not convincing evidence in favor of an origin from the mucous glands.

In alveolar tumors, it is also stated, that there are diffuse areas suggesting an organizing bronchopneumonia. It is very doubtful, if much, indeed, if any, significance can be attached to such a finding. Lung tumors are usually of widespread distribution and as has been stated above in four of the five cases reported, there is an extension into the pulmonary parenchyma, the cancer cells apparently growing along the thickened alveolar walls replacing the alveolar epithelium and filling the alveolar spaces. The alveolar epithelium, not infrequently assumes a cuboidal form and may be piled up in several layers so that it is sometimes difficult to determine whether a small group of cells represents regenerating alveolar epithelium or a portion of the neoplasm. These findings are present in many cases and may lead to the false interpretation of alveolar origin.

The difficulties of making an exact histogenetic diagnosis have been mentioned by others (Paessler,⁷ Adler⁹), but to find, within a single tumor, areas resembling the generally accepted cases of all three varieties throws further doubt on the possibility of determining the exact origin in the large majority of carcinomas of the lung. The five cases in this series are all of bronchial origin, but it is difficult or impossible to determine the more minute histogenesis except in Case 5 (a keratinizing squamous cell tumor), which apparently arose in the bronchial mucosa.

Relation of Irritation to Neoplastic Growths—The emphasis that many authors have placed on chronic irritation from inflammatory processes as an etiologic factor in the causation of lung tumors has been previously mentioned. That carcinomas may result from certain types of chronic irritation is now generally conceded, and this phase of the subject will not be entered on except to mention the well known tumors following roentgen-ray dermatitis, the kangri cancers, and the chimney sweep cancers. In addition to these instances of definite association between irritation and the production of cancer there are other instances suggestive of such a relationship as in pipe-smoking and cancer of the lip, and the papillomas of the bladder in anilin dye workers.

Although considerable evidence has accumulated in favor of a causal relationship between chronic irritation and cancer, much less attention has been paid to the possibility of a neoplastic change being induced by an acute inflammatory process

The five primary bronchial carcinomas reported in this paper have occurred in a comparatively short time in a series of twenty-nine carcinoma cases among a total of 375 necropsies. Although the series is small, these figures (13.8 per cent of all necropsies and 17 per cent of all carcinoma cases) are strikingly high in comparison to those usually given. In these cases there is a preceding history of chronic inflammation of the respiratory tract in two cases, a definite history of influenza three months prior to the onset of the present illness in one case, and histologic evidence of preceding inflammatory processes, including hyperplasia and metaplasia of the bronchial lining epithelium and peribronchial perivascular areas of fibrosis, in all cases.

This association of pulmonary infection and lung tumors has been emphasized in studies of tumors in mice. Tyzzer⁴³ reported a series of eighty-three tumors occurring in seventy mice, of which 62 per cent were primary in the lungs, and fifty-two of the 70 mice, or 74 per cent, had primary lung tumors. Slye⁴⁴ found that one-third of all tumors of mice were primary in the lungs. Both Tyzzer and Slye observed evidence of preceding inflammation in many cases, and concluded that these tumors arose in areas of inflammatory hyperplasia.

The possible origin of these tumors in areas of inflammatory hyperplasia is particularly interesting in connection with the recent epidemic of influenza. In this disease a striking picture of epithelial proliferation is seen. The trachea, bronchi, bronchioles, and alveoli frequently show evidence of active epithelial proliferation running parallel to a widespread organizing process. In most cases the proliferation consists in a replacement in a more or less normal manner of the epithelium destroyed by the inflammatory process. In some cases, however, irregular cell nests extend into the surrounding newly organized area, producing a picture histologically indistinguishable from that seen in an infiltrating malignant epithelial new growth (Fig. 15). In discussing these regenerative changes, Winternitz, Wason and McNamara,⁴⁵ in their report on the pathology of influenza as seen in the recent epidemic, suggested the probability of an increase in lung tumors on the basis of the epithelial overgrowth observed.

43 Tyzzer, E. E. A Series of Spontaneous Tumors in Mice, with Observations on the Influence of Heredity on the Frequency of Their Occurrence, *J. M. Research* **21** 479, 1909.

44 Slye, M., Holmes, H. F., and Wells, H. C. The Primary Spontaneous Tumors of the Lungs in Mice, *J. M. Research* **30** 417, 1914.

45 Winternitz, M. C., Wason, I. M., and McNamara, F. P. The Pathology of Influenza, Yale Press, 1920.

The difference in the atypical epithelial proliferation in influenza and the comparatively orderly repair after lobar pneumonia is marked. The difference may be due to several factors—the site of the injury, the character and degree of the injury, and its repetition during the course of the disease. These differences have an analogy in the experimental study (Winternitz, Smith and McNamara⁴⁶) of epithelial regeneration after intrabronchial insufflation of hydrochloric acid in rabbits. The insufflation of a solution of hydrochloric acid causes necrosis of the tissue which varies with the amount and concentration of the acid used. With weak solutions the epithelium alone may be destroyed and it is quickly regenerated in a comparatively orderly manner. If the injury has destroyed the lining epithelium, the basement membrane, and a portion of the surrounding structures, the regeneration takes place in a very different manner and an interesting picture is produced. The normal repair consisting of a single layer of cells, is seen in those areas in which the basement membrane is intact, but when the regenerating epithelium reaches areas in which the necrosis has extended through the basement membrane, the epithelial cells pile up and penetrate the necrotic portions of the subjacent pulmonary parenchyma. Not only does the newly formed epithelium grow along the intact basement membrane and into the surrounding parenchyma in areas where the basement membrane is destroyed, but the proliferating cells extend over the dead epithelial membrane lying free in the lumen. Subsequently, this necrotic tissue may become organized with the formation of definite polypoid structures. With more marked pulmonary injury in which the acid has destroyed wide areas of lung tissue, the proliferation is more marked. The newly formed epithelial cells apparently extend out along the scaffolding of the necrotic alveolar walls, and in some areas the irregularity of the epithelial proliferation within the newly organized areas of destroyed pulmonary parenchyma presents a histological picture resembling a malignant epithelial tumor. The reparative changes in lobar pneumonia are analogous to those following slight chemical injuries, such as after weak acid, whereas the epithelial regeneration following influenza is comparable to that which follows a severe injury such as is produced by stronger acid. The association of the different degree of regeneration with the destruction of the basement membrane is interesting and agrees with the observation of Haythorn, that true squamous cell metaplasia of bronchial epithelium is always associated with destruction of the membrana-propria.

46 Winternitz, M. C., Smith, G. H., and McNamara, F. P. Epithelial Proliferation Following the Intrabronchial Insufflation of Acid, *J. Exper. M.* **32**: 205, 1920.

The ultimate fate of the exuberant epithelium produced by the intra-bronchial insufflation of hydrochloric acid in rabbits is not known, as only the earlier stages have been studied. It is probable that the epithelial proliferation both in influenza and in experimental acid insufflation, will undergo regressive changes in the majority of cases or else remain quiescent and appear as isolated epithelial structures in a dense scar. However, it is not improbable that in a small percentage of these cases the activity of the epithelium may develop into a true neoplastic process. It is quite conceivable, as suggested above, that the relatively high incidence of carcinoma of the lungs in this hospital during the past two years may be referable to postinfluenzal lesions even though the clinical history in a majority of cases was not definite as regards an antecedent infection.

CONCLUSIONS

1 Classification of carcinomas of the lungs, either on a basis of gross pathological or histological structure, is difficult and unsatisfactory. The majority of cases are seen in a late stage, and while there are morphologically several definite types, including mucus secreting adenocarcinomas, and prickly cell tumors, the majority of cases do not fall into well defined groups but present striking variations in structure in different parts of a single tumor which are especially confusing.

2 The exact origin of tumors of the lung from the bronchial mucosa, the bronchial mucous glands, or the alveolar epithelium, is in general impossible to determine with certainty, but gross and microscopic evidence point strongly to a bronchial origin for most of the tumors. All five of the cases herewith reported very certainly originated either in the bronchial mucosa itself or in its glands.

3 According to composite statistics compiled prior to 1917, carcinoma of the lungs was found in approximately 0.36 per cent of necropsies and 1 per cent of all carcinomas. During the past three years five cases have been found at the New Haven Hospital in 375 necropsies, or 1.38 per cent of all necropsies, and 17 per cent of carcinomas. Whether these figures indicate a real increase in the incidence of carcinoma of the lung is doubtful, but the observation is at least suggestive.

4 The lungs in fatal cases of influenza occurring in the recent epidemic showed very regularly severe damage to the bronchial and alveolar epithelium, often associated with striking and atypical epithelial proliferative changes resembling early carcinomas. Such lesions suggest the possibility of a causal relation between influenzal infection and the recent apparent increase in carcinoma of the lungs, indicated by the figures given above.

EXPERIMENTAL DIABETES INSIPIDUS*

PERCIVAL BAILEY AND FRÉDÉRIC BREMER

BOSTON

BRUSSELS

INTRODUCTION AND HISTORY

It has been known for a long time that tumors and other pathologic lesions in the region of the sella turcica are attended by far-reaching pathologic alterations in other parts of the body. For some time also it was a matter of dispute among clinicians as to whether the symptoms were due essentially to lesion of the pituitary gland or of the immediately adjacent base of the brain (Erdheim¹). Recently this controversy has arisen anew in the contradictory articles of Leschke² and Marañón³.

In 1907 Paulesco⁴ opened the way to a solution of the question by developing a method of operative approach to the region of the sella. By this method, perfected by Cushing and developed by him and his associates, operative procedures on the pituitary gland became relatively easy.

Cushing⁵ was the first to note, after experimental lesions of the pituitary in dogs, the development of a syndrome identical with that known clinically as adiposogenital dystrophy. The syndrome in his experience followed most frequently division of the pituitary stalk, an observation recently confirmed by Bell⁶. The same dogs had a persistent polyuria. These results are usually attributed to deficiency of the secretion of the pituitary gland: the adiposogenital dystrophy to deficiency of the anterior lobe, the polyuria, to deficiency of the posterior lobe secretion. The latter opinion concerning the polyuria has been supported by the effects of subcutaneous injection of pituitrin and is widely accepted.

Shortly afterwards Aschner⁷ operating by the buccal route on dogs, denied the possibility of producing adiposity in adult animals by removal of the gland. On the other hand, he reported, after hypophysectomy in young dogs, the production of adiposity, genital aplasia, high

*From the Laboratory of Surgical Research, Harvard Medical School, and Surgical Clinic, Peter Bent Brigham Hospital.

1 Erdheim Sitzungsber d k Akad d Wissensch, math naturw Cl, Wien, Abt 3 **113** 537, 1904.

2 Leschke Ztschr f klin Med **87** 201, 1919.

3 Marañón Nuevas orientaciones sobre—de la Diabetes Insípida, 1920.

4 Paulesco L'hypophyse du cerveau, 1907.

5 Cushing The Pituitary Body, 1912.

6 Bell The Pituitary, 1920.

7 Aschner Arch f d ges Physiol **146** 1, 1912.

adrenalin tolerance, and general underdevelopment, a syndrome identical with that known clinically as pituitary infantilism. These symptoms he ascribed to lack of the pituitary secretion. The occasional occurrence in adult animals of other symptoms noted by Cushing, such as cachexia, subnormal temperature, adiposity, genital atrophy and death, he thought to be due to gross lesion of the brain. This latter assumption was purely gratuitous as any one who has examined a brain after a temporal pituitary operation must realize, even when done by a much less skillful surgeon than Cushing.

Aschner was vigorously criticized by Biedl⁸ for assuming such a totally unknown hypothetical nervous center as a cause for the symptoms. And there is an evident lack of coordination in Aschner's conclusions in that he attributed the genital atrophy in young animals to lack of pituitary secretion and the identical condition in adult animals to a lesion of the base of the brain.¹ Indeed, at that time so little was known concerning the connections and functions of the part of the brain just above the pituitary, that the idea that a lesion in this region so small as to escape naked-eye examination could cause such widespread results as polyuria, genital atrophy, adiposity, cachexia, and even death must have seemed to any one absurd. But though at the outset confusion and contradiction existed in the physiology of the pituitary, it came to be generally accepted that adiposogenital dystrophy and polyuria were due to an hypophysial lesion.

Since that time, however, some experiments have been reported, acute and rough though they were, which seem to indicate that in the base of the brain just over the pituitary is a very important center, intimately connected with the entire visceral nervous system. Lesion or stimulation of this region, known to anatomists as the hypothalamus, is said to be followed by dilatation of the pupil (Karplus and Kreidl⁹), a disturbance of heat regulation (Isenschmidt and Krehl,¹⁰ Ott,¹¹ Leschke,² Barbour¹²), glycosuria (Aschner¹³), polypnea (Garrelon and Langlois¹⁴) and many other symptoms.

The anatomic relations of the hypothalamus are still imperfectly understood, but it is known to be closely related to the olfactory and gustatory (Herrick¹⁵) systems, which are highly specialized parts of the visceral nervous system. There is also some evidence of

8 Biedl. *Innere Sekretion*, 1912.

9 Karplus and Kreidl. *Arch f d ges Physiol* **129** 138, 1909.

10 Isenschmidt and Krehl. *Arch f exper Path u Pharmakol* **70** 109, 1913.

11 Ott. *Fever, Its Thermotaxis and Metabolism*, 1915.

12 Barbour. *Physiological Reviews* **1** 295, 1921.

13 Aschner. *Berl klin Wchnschr* **53** 772, 1916.

14 Garrelon and Langlois. *J d physiol* **15** 566, 1913.

15 Herrick. *J Comp Neurol* **15** 375, 1905.

its connection with the general visceral system (Brugsch, Dresel and Lewy¹⁶)

Camus and Roussy¹⁷ were the first to undertake to control systematically the physiology of the pituitary by puncturing the base of the brain. Already in 1913 they had reported to the Société de biologie that they were able to produce transitory polyuria by puncturing the hypothalamus of dogs through the sphenoid bone with a heated drill. In one dog a permanent polyuria and in addition adiposogenital dystrophy had resulted. However, no microscopic examination was reported and it was often evident on gross examination that a lesion of the pituitary also existed. Especially was this true in the more interesting of their dogs, the one with a permanent diabetes insipidus with testicular atrophy. The report stated that the lesion lay in front of the infundibulum. This was much dilated, and the pituitary itself weighed only 20 cg. They concluded that the polyuria was due to a lesion of the gray matter of the tuber cinereum in the region of the infundibulum. They were not affirmative about the adiposogenital syndrome.

From time to time since then they have reported further experiment,¹⁸ and have shown conclusively that the polyuria does not depend on a lesion of the hypophysis by producing it in a dog from which the hypophysis had been previously removed. The removal of the hypophysis was followed by a transitory polyuria. More than a month later, after the polyuria had subsided, a second polyuria of equal intensity was produced by puncture of the tuber cinereum. Their conclusions have been supported by Houssay¹⁹ without adding any new information. It is interesting, in view of the results given later in this paper, that he was unable to produce these symptoms in operating by the temporal route, probably because of unfamiliarity with the operative procedure.

Recently Camus and Roussy²⁰ in demonstrating two dogs with adiposogenital dystrophy to the Société de neurologie admitted that a lesion of the pituitary was probably present, although they brought strong arguments to show that it was not essential. Indeed in operating by their method it is difficult to avoid puncturing the pituitary, which lies directly under the hypothalamus.

Since it seemed difficult to arrive at a decision by this method with its almost necessary concomitant injury of the hypophysis, we

16 Brugsch, Dresel and Lewy. *Ztschr f exper Path u Therap* **21** 358, 1920.

17 Camus and Roussy. *Compt rend soc Biol* **75** 483, 1913.

18 Camus and Roussy. *Endocrinology* **4** 507, 1920, *Compt rend soc Biol T* **83** 1578, 1920.

19 Houssay. *Endocrinology* **2** 94 1918, *Compt rend soc Biol* **81** 381, 1918.

20 Camus and Roussy. *Rev neurol* **27** 1113, 1920.

determined to attack the hypothalamus by the temporal route, which allows an exposure of the entire region, with intent to study in detail the resulting polyuria. The lesion can be produced where desired, and the pituitary, being in plain view, carefully avoided. Needless to say, no trauma whatsoever is produced by the slight cerebral dislocation necessary for this exposure.

The present report is concerned with a series of such experiments, with especial reference to the resulting polyuria, leaving the discussion of the adiposogenital syndrome for a later communication.

METHODS

Dogs were used throughout our experiments. The technic of the temporal operation has been described in detail by Crowe, Cushing and Homans²¹. After the first few operations this procedure was modified somewhat to suit our purposes. The curved incision used by Bell⁶ was found adequate. On the right side a subtemporal decompression was done as usual. On the left side we adopted practically the procedure of Sweet and Allen²².

The superficial muscles are drawn down below the zygoma. A horizontal incision is then made through the periosteum along the middle of the zygoma for its entire length. The periosteum is stripped off the zygoma upward and downward. The zygoma is resected. From the middle of this horizontal incision another is made through the temporal muscle slanting upward and backward in the direction of the muscle fibers. In the upper part this latter incision is carried down to the skull. In the lower part the coronoid process of the mandible intervenes and must be removed before the incision can be extended downward. The temporal muscle is stripped from the skull and held to right and left with retractors. The bone is then trephined and the operation continued as usual. This procedure permits of a very low approach, direct access to the pituitary with a minimum of retraction of the brain and a perfect closure.

The pituitary region having been brought into view it is easy to recognize optic nerves, stalk, pituitary, internal carotid, etc., and to make lesions in any part of the hypothalamus at will. For making the lesion in the brain at first we used a right-angled knife which was slid between the pituitary and base of the brain, the point turned upward, thrust into the brain and then drawn to one side. After cutting the circle of Willis, however (Dog 12), it was discarded for a sharp right-angled probe. After the first three dogs also we contented ourselves with a much smaller lesion.

A few buccal operations were done following the technic described by Aschner,⁷ and also a few punctures after the manner of Camus and Roussy by boring through the sphenoid with a dental drill, and puncturing the brain with a sharp probe.

The brains were removed as described by Crowe, Cushing and Homans after fixation *in situ* by carotid injection of formalin, with

²¹ Crowe, Cushing and Homans. *Johns Hopkins Hosp. Bull.* **21**: 127, 1910.

²² Sweet and Allen. *Ann. Surg.* **57**: 485, 1913.

the exception of Nos 10, 17 and 22 In these cases the body of the sphenoid was removed through the mouth as in a buccal operation and the dura carefully cut around the edge of the bony defect The brains were then removed as before from above, the dura remaining attached to the entire hypothalamic region

The hypothalamus was removed in one block, from the optic chiasm to the pons, with the pituitary attached The block was embedded in celloidin, cut in 20 microns serial sections, and stained with van Gieson's mixture In Dogs 7, 13, 15 and 16 the pituitaries were cleanly severed from the brain, embedded in paraffin and cut 6 microns in series for more careful study since in these cases there was some question of injury to the pituitary In Dog 10, every fourth section of the brain was stained with thionin

PROTOCOLS

The location of the lesion in each dog except the first two may be seen by referring to Plate 1

Dogs 1 AND 2—Adult dogs No 1, partial anterior lobe removal, verified histologically No 2, posterior lobe removal, verified histologically Neither dog had polyuria At necropsy the remaining pituitary tissue normal histologically in each case Base of brain not sectioned

Dog 3—Male, adult, 132 kg Puncture by temporal procedure, November 3, previous average output 300 cc Polyuria second day Maximum 1,500 cc, November 12 Apathy, drowsiness, arched back, convulsions, hypothermia Died in convulsion November 15

Necropsy—General examination negative, microscopically, lesion a puncture wound of the hypothalamus extending upward and forward to a depth of about 3 mm Lesion directly back of the stalk of the hypophysis and slightly to the right of the midline Hypophysis microscopically and macroscopically normal Meninges normal Testis showed acute atrophy of spermatogenic elements, rarely a spermatozoa or a spermatid is seen and they are absent in many tubules where the wall is composed of Sertoli cells and a few scattered spermatogonia, the spermatocytes show signs of degeneration in form of increased fat content Suprarenal, pancreas, liver and kidney histologically normal

Dog 4—Male, adult, 25 kg Temporal puncture November 18, previous average output 500 cc Polyuria the same day, 1,030 cc Coma, convulsions, death on the third day

Necropsy—Subcutaneous infection on left side Meninges normal General findings negative Clean knife cut transversely across hypothalamus just back of stalk of hypophysis extending upward to a depth of 4 mm Hypophysis intact No signs of infection of the meninges

Dog 5—Male adult, 118 kg Temporal puncture November 30 Previous average output 700 cc Glycosuria first day Polyuria the third day Maximum 1,200 cc on sixth day Apathy, drowsiness, arched back Polyuria ceased the ninth day Killed December 21

Necropsy—General findings negative Microscopically two knife cuts, one into the mamillary bodies, the other just back of them extending upward about 3 mm Hypophysis intact

Dog 6—Male, adult, 12 kg Temporal puncture December 2 Previous average output 450 cc Polyuria the second day Maximum 1,500 cc the third day Polyuria ceased the seventh day Aschner buccal operation,

December 18 Puncture in front of hypophysis No polyuria, no symptoms except left hemianopsia Killed January 31

Necropsy—General findings negative Meninges normal Two lesions, one a small puncture wound just behind and to the right of the hypophysis and extending to a depth of about 2 mm, the other a much deeper one extending from the optic chiasm to the thalamus Pituitary intact

Dog 7—Male, puppy, 8 months, 134 kg Average output 200 cc Aschner buccal operation March 1 Dura not opened Curved dental probe slid around hypophysis and thrust into brain above it No bleeding Marked polypnea Polyuria the first day 960 cc, apparent recovery Died the third day of meningitis

Necropsy—Meningitis Puncture wound extending through the left mamillary body into midbrain Pituitary infected

Dog 9—Male, adult, 15 kg Temporal puncture, January 18 Previous average output 350 cc Polyuria the second day Maximum the third day, 1,160 cc Ceased the fifth day Killed February 2

Necropsy—General findings negative Superficial lesion just back and to the right of the stalk Hypophysis intact (Plate 2)

Dog 10—Male, adult, 129 kg Average output 450 cc Temporal puncture January 7 Enormous thirst Polyuria the first day Maximum the fifth day, 3,800 cc Permanent diabetes insipidus Arched back and apathy the first days Insidious and progressive drowsiness, obesity and genital atrophy Kidneys denervated April 16 Transitory increase of polyuria and persistence later of the diabetes Intramuscular injection of 4 cc epinephrin May 10, 9 a m No glycosuria Killed May 10 in evening Weight 20 kg

Necropsy—Enormous accumulation of fat generalized Atrophy of external genitalia and testes Thyroid not enlarged Meninges normal V-shaped lesion in hypothalamus just back of the stalk of the hypophysis The posterior limb extended upward through the anterior margin of the mamillary bodies, the anterior slanted forward over the stalk to a depth of 3 mm Anterior to the incision the cells of the tuber show chromatolytic changes Behind the incision the nerve cells are normal The entire pituitary was present The posterior lobe had been detached from the infundibulum but was normal and its blood supply intact The entire anterior lobe was present, its cells stained normally and its blood supply intact Testes show an extensive atrophy of the spermatogenic elements The spermatozoa are absent as well as most of the spermatids Many pyknotoses are seen in the spermatogonia There is slight sclerosis of the walls of the tubules, very slight interstitial sclerosis, marked sclerosis of the albuginea, and extreme vascular sclerosis without endarteritis (Plate 3) The cells of the interstitial gland contain an abnormally large amount of fat which is collected in large globules Suprarenal normal, lipoid content of cortex unaltered Thyroid, parathyroid, pancreas, liver and kidney histologically normal

Dog 12—Male, adult, 12 kg Temporal operation January 25 Operation abandoned without puncture because of profuse hemorrhage from circle of Willis No polyuria Marked sleepiness, contrasting with an excellent general state and which persisted until the animal was sacrificed No choked disk Killed February 15 Extensive pachymeningitis hemorrhagica interna

Dog 13—Male, adult, 134 kg Average output 300 cc Temporal operation, February 7, abandoned because of hemorrhage from cavernous sinus No symptoms Removal of hypophysis by Aschner buccal procedure, February 21 Polyuria the first day, 1000 cc Died February 23, with temperature of 109 F

Necropsy—General findings negative Very little blood in subarachnoid space No signs of infection Pituitary entirely gone Extensive lesion of the tuber cinereum which was not entirely destroyed but showed contusion and hemorrhage to a depth of 4 mm

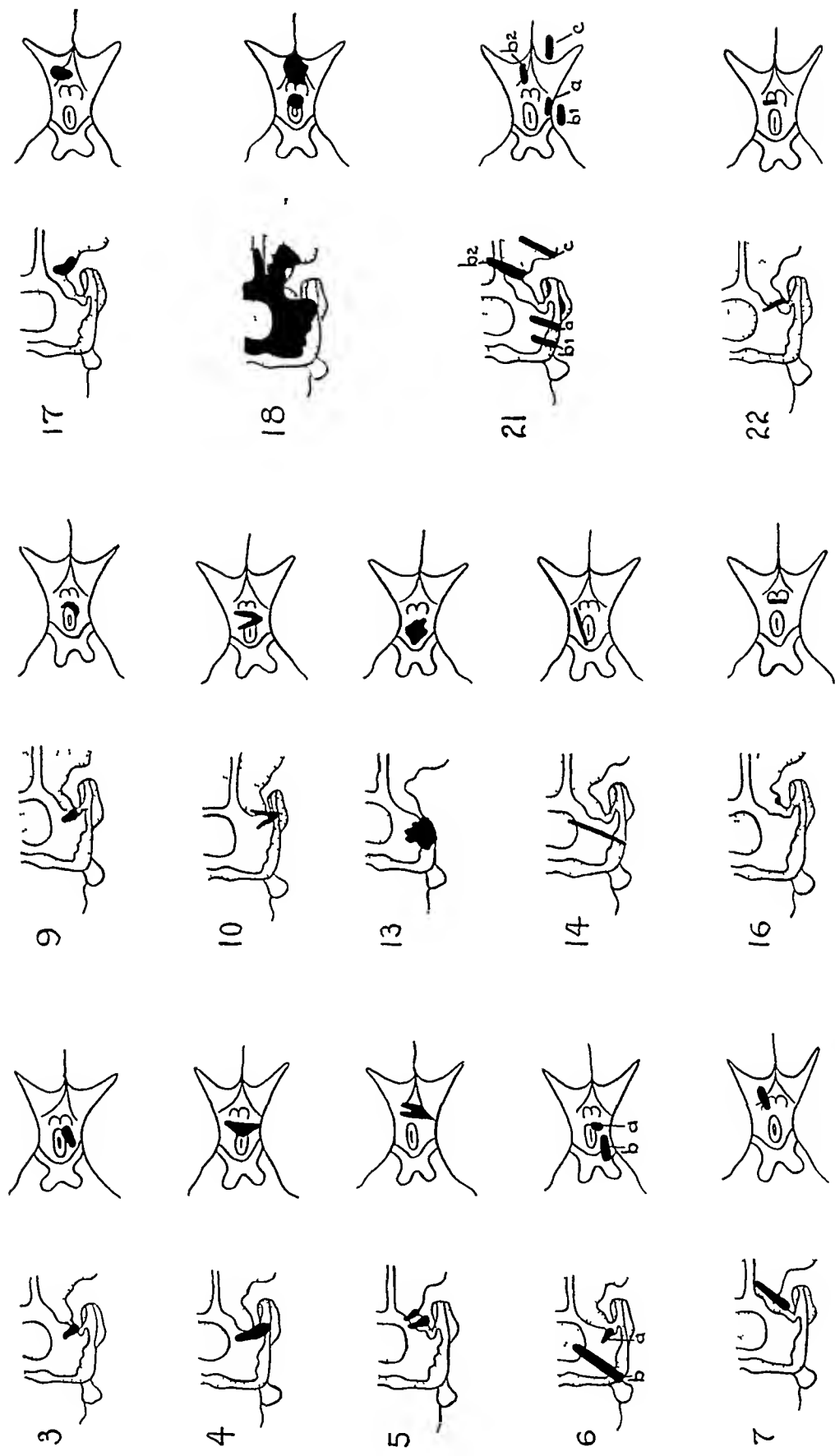


Plate 1 —Diagrams of lesions The lesions are in black, projected on a median sagittal section and on the base of the brain

Dog 14—Male, adult, 196 kg Chronic nephritis Irregular polyuria average 800 c c Puncture through sphenoid with a dental drill Immediate polyuria which lasted twenty-four hours Polyuria the third day Maximum 2300 c c Ceased the seventh day Traces of glucose the third day Killed April 11

Necropsy—Small contracted kidneys with capsule adherent Minute puncture wound directly in front of the stalk of the hypophysis extending upward and backward to a depth of 15 mm (Plate 2) Hypophysis intact Kidney showed subacute nephritis

Dog 15—Male, adult, 107 kg Temporal operation February 19 Strong trace of glucose first day No polyuria No other symptoms Killed March 11

Necropsy—General findings negative No lesion of hypothalamus could be found Hypophysis intact

Dog 16—Male, adult, 136 kg Average output 400 c c Temporal operation, February 26 Strong trace of glucose the first day Polyuria the second day Maximum 1,700 c c on same day Ceased the eighth day Killed March 15

Necropsy—General findings negative Meninges normal Superficial lesion at anterior border of mamillary bodies Hypophysis intact

Dog 17—Male, adult, 10 kg Temporal puncture March 29 Immediate polyuria No polyuria Died the first day

Necropsy—Considerable contusion of left temporal lobe Lesion in left cerebral peduncle Hypophysis intact

Dog 18—Female, adult, 10 kg Temporal operation, April 5 Slight polyuria Strong glycosuria Died April 6 in coma

Necropsy—Intraventricular hemorrhage

Dog 21—Male, adult 12 kg Transsphenoidal puncture (a Plate 1) April 23 No polyuria Marked glycosuria April 26, two similar punctures (b, b, Plate 1) Marked polyuria No polyuria Slight glycosuria May 4, one similar puncture further back (c, Plate 1) No polyuria Marked glycosuria Killed May 10

Necropsy—Meninges normal Four puncture wounds of the brain were found, only one of which (a) struck the optopeduncular region and was far lateral to the midline One of the other three punctures struck the hypophysis producing a hemorrhage into the interlobar cleft

Dog 22—Male, young adult, 10 kg Average output 400 c c Temporal puncture April 29 Polyuria the second day which did not subside, maintaining an average of 1,100 c c Kidneys denervated May 15, weight 152 kg Subcutaneous infection very ill When the infection subsided the polyuria reappeared May 25, weight 11 kg, general condition improving rapidly June 5 injection of 4 c c epinephrin intramuscularly No glycosuria Killed June 10, weight 132 kg

Necropsy—Moderate generalized deposit of fat Evidence of previous extensive intramuscular infection of lumbar region Meninges normal Lesion in hypothalamus at anterior border of the mamillary bodies Hypophysis intact Testis showed acute atrophy, spermatozoa almost completely absent, spermatids absent in many tubules, the remaining ones with increased fat content, swollen and degenerating Increased amount of fat in the interstitial cells also Kidney, suprarenal, thyroid, pancreas and parathyroid histologically normal

Dog 23—Female, adult, weight 146 kg Average output 450 c c, sp gr 1026 June 1, kidneys denervated Polyuria reaching a maximum of 900 c c June 3, output 760 c c June 4, temporal puncture, intake 1,800 c c, output 600 c c June 5 apathetic June 6, still apathetic, intake 1,525 c c, output, 997 c c June 7 cachectic, apathetic, with head drooping, arched back, profuse thick salivation, slow pulse and repeated typical epileptic attacks June 8, some-



Plate 2—Photomicrographs of approximately median sagittal sections of hypophysis and hypothalamus, $\times 14$. The sections are reversed. Lesions shown at \times . Dog 14 above, Dog 9 below.

what improved, fewer attacks June 10, much improved, injection of 4 c.c. epinephrin intramuscularly gave no glycosuria June 11 much improved, intake 800 c.c., output 850 c.c., sp. gr. 1.018 Found dead in the morning of June 20

STUDY OF THE SYMPTOMS

As an immediate result of the puncture we noted a sudden change in the respiratory rate which in one dog became a true polypnea lasting for twenty-four hours without other symptoms. In two cases also the animal gave a peculiar cry at the moment of puncture, both being nevertheless deeply narcotized at the time. We never observed any change in the rectal temperature within the first four hours.

1 *The Glycosuria*—This was noted in six cases and had the characteristics of Claude Bernard's puncture glycosuria, the exact mechanism of which is still unknown. Stewart and Rogoff,²³ confirming others, have shown recently that it may be produced as well in animals whose adrenals have been removed. We made no attempt to determine the exact mechanism of its production in our animals.

In four of these dogs there was also a polyuria and it is interesting to note that in Dog 14, who had a chronic nephritis with marked retention of urea (nonprotein nitrogen 100), the trace of glucose appeared only on the third day along with the polyuria. It may be seen from the sketches (Plate 1, 5, 14, 16, 18) that in each case the hypothalamus was involved in some way.

Two dogs (15 and 21) had glycosuria without polyuria. Dog 21 was punctured three times by the buccal route and each time had glycosuria. The lesions in this animal were all in the outskirts of the hypothalamic region and in Dog 15 no lesion could be found. It seems that the glycosuria does not depend upon so localized a region as the polyuria, and its production is known to depend very greatly on the nutritional state of the animal.

2 *Hyperthermia*—One dog (No. 13) following ablation of the pituitary by the buccal route, had a temperature of 109 F. the second day, and died. The lesion in this dog who had also polyuria but no glycosuria affected extensively the tuber cinereum.

It has been shown by Ott, Isenschmidt and Krehl and others that the hypothalamus is the most important thermic center in mammals; thermic regulation and the possibility of inducing fever is abolished after section of the brain stem just behind it. Puncture of this region is followed in the rabbit (Ott¹¹) by an immediate and extreme hyperthermia. The same hyperthermia, usually fatal, may follow operations on the pituitary region in man. That we did not observe it more frequently in our experiments is probably due to the small size of the lesion.

²³ Stewart and Rogoff. *Am. J. Physiol.* 46:90, 1918.



Plate 3—Photomicrographs of sections from the testis of Dog 10 $\times 70$
The upper is stained with scarlet red, the lower with hematoxylin and eosin

3 *Polyuria*—In order to study the polyuria the dogs, kept in metabolism cages, were given free water supply and daily 1½ pounds of carefully weighed lean meat. The intake of water, output of urine, and the freezing point (Δ), specific gravity and total chlorids of the urine were charted. The presence of glucose and acetone in the urine was tested for before and after operation.

The daily output of urine on this regime in these animals, all adult, middle-sized dogs, varied from 200 to 500 c c, but was very regular for each dog. Much more constant than the output were the freezing point and specific gravity. The freezing point varied between -3 and -4 in the different dogs, but each dog had a characteristic

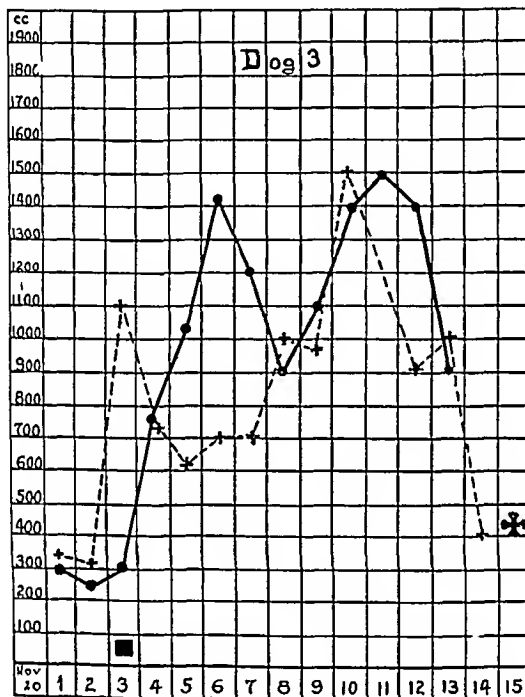


FIG. 1 +---+ Intake ●—● Output ■ Operation

freezing point of an amazing constancy, fluctuating only two or three tenths of a degree. This is due to the fact that the dog eliminates urea to the maximal concentration (Ambard). After a few days of this regime poor in chlorids, the dog eliminates a minimal quantity of chlorids, from 0.8 to 1 gm, in twenty-four hours. They consist partially of potassium chlorid but for the sake of simplicity are evaluated in sodium chlorid. As has already been said, the dogs recovered completely within two hours after the operation. Most of them drank the day of operation and all, except the comatose ones and those operated by the buccal route, ate their entire ration the following day. Twelve of the fifteen punctured dogs had a polyuria.

Three dogs which were punctured had no polyuria (Nos 15, 17 and 21). In Dog 15 no lesion could be found, in Dog 17 the only

lesion was in the midbrain and in Dog 21 the lesions were all around the edge of the optopeduncular region. Besides Dog 12, submitted to the same operation but not punctured because of a profuse hemorrhage from the circle of Willis, had no polyuria.

The polyuria began for five dogs the first day, for six the second day and for one the third day. This last dog had a chronic nephritis with retention. Though there was apparently long latency of the effect of the puncture on the regulation of water, in three cases where the polyuria did not appear until the second day there was already on the first day a considerable increase in the intake of water as compared

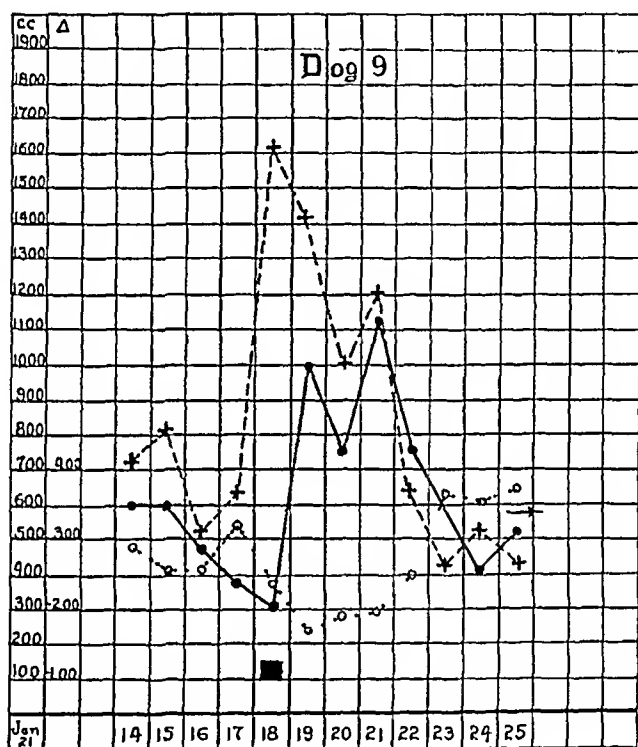


Fig 2 +---+ Intake, ●—● Output o o Δ ■ Operation

with the preceding days (Fig 1, 2 and 3). Furthermore, e.g. in Dog 10, there was great disproportion between the intake and output on the days immediately following the operation, on the first day an intake of 2,500 cc, and an output of 900 cc, on the second, an intake of 3,600 cc and an output of 2,400 cc (Fig 3). The same disproportion may be seen in Figure 2. This extreme discrepancy does not last more than two or three days. Soon an adjustment takes place, sometimes even the reverse discrepancy existing for a short time (Fig 2). Whatever may be the cause of this early difference, the fact seems to be very important: it shows that polydipsia may precede the polyuria and is not simply consecutive to it.

Camus and Roussy do not mention this fact and their published curves do not show it. We have noticed that the dogs operated on by the buccal route did not drink the first day, which may explain their failure to observe this phenomenon. But these authors did remark various discrepancies between the intake and output later in the polyuria and employed the designation "trouble with the regulation of water" and they insist on the fact that the polydipsia, which they think nevertheless to be consecutive to the polyuria is enormous, inordinate and seems at times to be out of proportion to the needs of the organism.

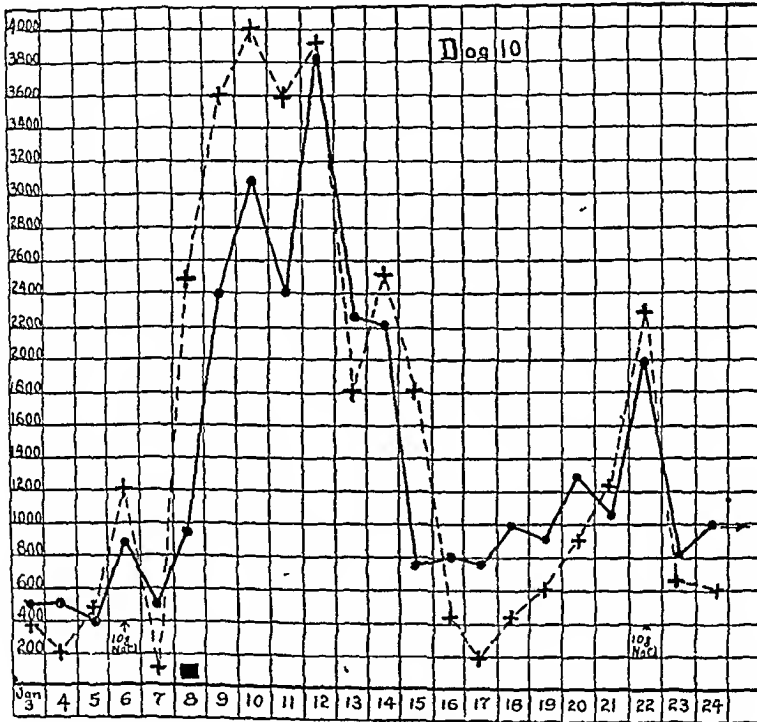


Fig 3 +---+ Intake ●—● Output ■ Operation

On the other hand, it is well known that the polyuria may appear (e g in comatose animals) and persist without any intake of water. We shall discuss later the significance of these facts.

In all but three dogs the polyuria lasted only from six to ten days and attained its maximum of from 1,000 to 3,000 cc on the third or fourth day as in the experience of Cushing²⁴ and of Camus and Roussy.

In the ten dogs with a polyuria in which a clean-cut lesion was found it was always located in the hypothalamus just back of the stalk of the pituitary, except in Dog 14 where it was just in front. Of the remaining two, one (No 13) had an extensive lesion of the tuber and the other (No 18) a hemorrhage into the third ventricle.

24 Cushing Boston M & S J 168 901, 1913

with considerable trauma to hypothalamus and midbrain. It should be remembered that in the three dogs who were punctured but had no polyuria, no lesion of the hypothalamus was found but various lesions all around this region (including in Dog 21 the pituitary). Thus both the positive and negative results point to the same conclusion, namely, that the polyuria depends on a very small area just above the stalk of the pituitary known usually as the tuber cinereum.

4 *Permanent Polyuria*—In three dogs the polyuria was permanent and had, as we shall see, all the characteristics of diabetes insipidus in man. These dogs presented other interesting symptoms. No. 3

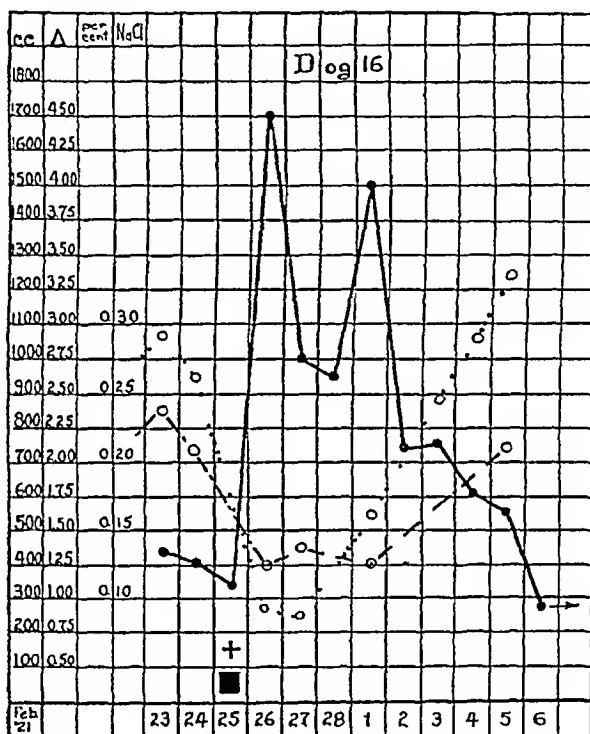


Fig 4 ○---○ NaCl per cent, ●—● Output, ○- -○ Δ ■ Operation + Sugar

having progressive cachexia, hypothermia, and convulsions, Nos. 10 and 22 had typical adiposogenital syndrome. Such an association of symptoms is not a coincidence but is well explained by the lesions because in these dogs the lesion in the tuber was a more important one, although macroscopically very small. Those dogs having a still more extensive lesion of the tuber died very quickly as will be seen later.

In these polyurias the fall of the Δ , in other words, the total molecular concentration, was always more marked than the fall of the concentration of the chlorids (Table 1). The consequence was that the dogs during the days of excessive polyuria lost an appreciable quantity of chlorids, as much as 3 gm. in twenty-four hours. This

dissociation between the concentration of urea and other nonthreshold substances, and the concentration of chlorids is a banal one, as Cushny points out being found in all polyurias. It is explained in the modern theory of urinary secretion by impairment of the reabsorption of chlorids due to increased speed of flow in the tubules. In animals like the rabbit where there are other reasons for supposing a less developed power of reabsorption, any polyuria is accompanied by a marked increase in the percentage of chlorids in the urine (Cushny). After the polyuria had ceased it could be shown in three dogs that there was still a trouble of the regulation of water by the excessive polyuria provoked by the ingestion of chlorids.

TABLE 1—Dog 10

Date	Urine 24 Hours	Δ	NaCl, per Cent	Total NaCl	
1/ 3/21	500	—3.70	0.20	1.00	10 gr NaCl Operation
1/ 4/21	580	—3.40	0.18	1.04	
1/ 5/21	425	—3.90	0.23	1.035	
1/ 6/21	900	—3.60	0.92	8.800	
1/ 7/21	565	—3.95	0.46	2.56	
1/ 8/21	940	—0.45	0.18	1.69	
1/ 9/21	2,400	—0.55	0.10	2.40	
1/10/21	3,040	—0.95	0.11	3.344	
1/11/21	2,150	—0.75	0.082	1.76	
1/12/21	3,800	—0.95	0.052	1.976	
1/13/21	2,240	—1.40	0.09	2.016	
1/14/21	2,150		0.057	1.24	
1/15/21	670	—3.60	0.111	0.737	

Dog 10 after a week of extreme polyuria (as much as 3,800 c c) presented during four months, until sacrificed, a permanent and very regular polyuria. Dog 22 had also a permanent polyuria which we followed during more than a month. Previous to his puncture the daily output of Dog 10 was 470 c c with a Δ oscillating closely around —3.7. After the puncture the output was from 900 to 1,400 c c of a very pale urine and an average Δ of —2.2. Never was the previous Δ reached spontaneously. The constancy of this new concentration was such that the animal seemed to have adopted a new osmotic regulation and was in marked contrast to that observed in two nephritic dogs with polyuria which we had opportunity to observe. This constancy was very favorable for a systematic study, our aim being to verify that this polyuria had the characteristics of clinical diabetes insipidus. The same constancy was also present in Dog 22.

(a) *Influence of Deprivation of Food*—No appreciable influence was observed.

(b) *Influence of Deprivation of Water*—Dog 10 was restricted to 200 c c of water daily for two or three days. The output diminished only after the second day and then but slightly. The Δ reached

the neighborhood of -3 In one experiment there was an output of 1,000 cc on the third day of thirst, and the animal had lost at least 2,000 cc water The blood, which was examined for us by Dr Hilding Berglund before and at the end of the experiment, showed no evidence of concentration or retention

(c) *Effect of Pituitary Extract*—The effect of subcutaneous injection of pituitary extract was very interesting because Camus and Roussy state that it has no effect on the permanent polyuria which they produced in their dogs The variations which they observed in the output after pituitary extract injections were of the same order

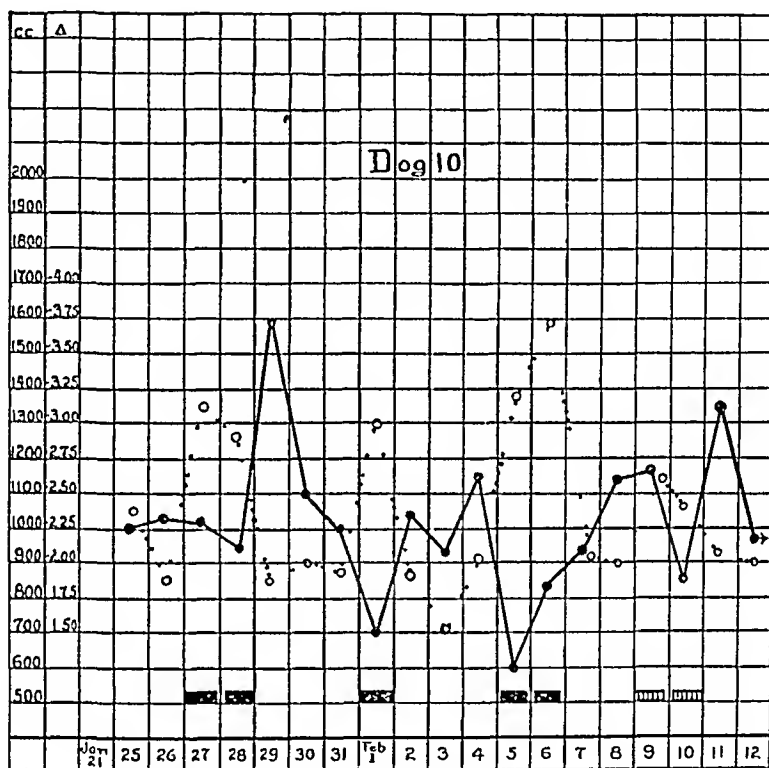


Fig 5 Showing the action of Pituitary Extract and of reduction of the intake
 ●—● Output ○ -○ Δ, ■ 2 cc Pituitary Extract in four injections ■ Intake reduced to 200 cc

as the daily variations The failure to discover such an effect was probably due to the fact that they did not employ repeated small doses nor observe the Δ or specific gravity

The effect of subcutaneous injection of pituitary extract (Parke, Davis & Co pituitrin, and Lederle) was constant and definite, but much more apparent in the Δ than in the output (Fig 5, Dog 10), it was the same in Dog 22 But the action, if we consider the dose employed and the necessity of repeated injections, is certainly less marked than in the average case of diabetes insipidus in man Pituitrin had no definite action on the polyuria of two nephritic dogs

(d) *Ingestion of Sodium Chlorid*—The addition of an excess of sodium chlorid to the diet always produced a considerable increase of the polyuria. Normal dogs, living on a diet poor in chlorids, when given 10 gm sodium chlorid with their meat consume it with avidity, have only a slight polyuria or none at all, and eliminate the first day from 6 to 8 gm of the salt to a concentration of from 0.9 to 1 gm per cent. This seems to be the maximal ability of the normal kidney to concentrate sodium chlorid as we have seen it in giving from 20 to 30 gm. The excess of sodium chlorid is eliminated in two, three or even four days.

Table 1 shows the effect of ingesting 10 gm of sodium chlorid on Dog 10 before the puncture polyuria. Quite different was the result on the fourteenth day of polyuria (Table 2), when the output rose from 1,050 to 1,970 cc. The concentration reached only 0.6, but 12 gm chlorid were eliminated the first day and the elimination ceased

TABLE 2—Dog 10

Date	Urine 24 Hours	Δ	NaCl, per Cent	Total NaCl	
1/20/21	1,300	—2.35	0.18	1.69	10 gr NaCl
1/21/21	1,050	—2.10	0.155	1.62	
1/22/21	1,970	—1.4	0.60	12.02	
1/23/21	780	—2.50	0.25	1.95	
1/24/21	700	—2.60	0.15	1.05	
1/25/21	1,000	—2.4	0.12	1.20	

the same day. This fact seems to us very interesting in view of the long prevailing doctrine that the polyuria of diabetes insipidus is a compensatory one due to the inability of the kidney to concentrate and the necessity for it to eliminate more water in order to perform its function of excretion (E. Meyer²⁵ and others). It is difficult to conceive that a reaction against a supposed defect could have a better final result than the normal kidney is able to attain. We have found curves showing the same acceleration of the excretion of sodium chlorid in diabetes insipidus of man in the papers of E. Meyer and of Kennaway and Mottram²⁶ given by these authors to show the inability of the kidney to concentrate!

The test was repeated twice on Dog 10 with the same result, namely the rapid elimination of the entire amount at a low concentration (from 0.33 to 0.45) with a marked increase of the polyuria. But it was possible to prove that the kidneys were perfectly able to concentrate normally the sodium chlorid by giving the salt on the second day of an experiment of thirst. Then the normal maximal

²⁵ Meyer. *Deutsch Arch f klin Med* **83** 1, 1905, *Deutsch Klin* **13** 282, 1911.

²⁶ Kennaway and Mottram. *Quart J Med* **12** 225, 1918-1919.

concentration of 1 gm per cent was reached and a Δ higher than before the operation (Table 3) A similar result was obtained where salt was given and pituitary extract injections with free water intake The results with Dog 22 were entirely similar

The polyuric action of chlorids is characteristic of diabetes insipidus (Oehme²⁷) Our nephritic dogs did not show it. Therefore, it is interesting that we found the same excessive polyuria and quicker elimination of sodium chlorid not only during the polyuria but, by employing larger doses, after an apparent return to normal in dogs having a transitory polyuria Dogs 5 and 6 a week after the cessation of their polyuria were given together with two normal dogs 30 gm sodium chlorid They had the first day a polyuria of 1,900 c c, whereas the normal dogs passed only 1,000 and 1,200 c c, and the elimination of the salt was much quicker Dog 5 already after

TABLE 3—Dog 10

Date	Urine 24 Hours	Δ	NaCl, per Cent	Total NaCl	
2/14/21	850	—2 1	0 130	1 105	10 gr NaCl
2/15/21	950	—2 3	0 130	1 235	
2/16/21	1,580	—1 90	0 45	7 110	
2/17/21	1,000	—2 4	0 224	2 240	
2/18/21	925	—2 5	0 215	1 988	200 c c H ₂ O 200 c c H ₂ O, 10 gr NaCl
2/19/21	565	—2 9	0 200	1 130	
2/20/21	750	—4 05	1 097	8 227	
2/21/21	1,300	—2 15	0 205	2 665	
2/22/21	1,000	—2 4	0 198	1 980	
2/23/21	1,020	—2 25	0 119	1 213	

the first day eliminated his normal chlorid Other salts than chlorid, other crystalloids, and the purine derivatives have little or no effect on the polyuria of diabetes insipidus (Oehme) We have verified this statement in our dogs for theobromine

(e) *The Effect of Fever*—Fever was not provoked experimentally but Dog 10 had a slight attack lasting some days following an injection abscess During this time the polyuria was greatly diminished and the concentration of the urine increased in spite of the fact that he did not eat his entire ration

The results of all these tests justify the conclusion that the persistent polyuria provoked in this manner has all the characteristics of diabetes insipidus in man and is therefore a true experimental diabetes insipidus

(f) *Effect of Denervation of the Kidneys*—This was a procedure of great interest because, so far as we know, it has never been done either in clinical or experimental diabetes insipidus The denervation

was performed on Dog 10 on the ninety-eighth day of his diabetes by Dr William Quinby whose experience with the procedure is well known²⁸ All the nerves accompanying the renal vessels on both sides were destroyed and the surfaces of the vessels as far as possible decorticated At this period, the polyuria, although constant, averaged only 750 c c with a Δ of -2.3 as against 470 c c with a Δ of -3.7 before the puncture

The denervation was immediately followed by the polyuria which usually ensues The maximal denervation polyuria observed by Dr Quinby in his experiments on normal dogs was 1,000 c c, an exceptional amount Clearly Dog 10, who passed regularly 1,300 c c during eight days following denervation had two superimposed polyurias, one due to diabetes insipidus and the other to vasomotor disturbance produced by the denervation

When the vasomotor polyuria had subsided after the usual delay the puncture polyuria continued until the animal was sacrificed, with the same characteristics The average output was 850 c c, the Δ and specific gravity remained low, the effects of pituitary extract and sodium chlorid ingestion were unaltered, and the kidney showed the same ability to concentrate under the influence of pituitrin or restriction of water A phenolsulphonephthalein test was done May 8, more than 75 per cent being excreted in two hours and ten minutes In the experience of Dr Quinby this number is normal for dogs

In Dog 22 the kidneys were denervated on the seventeenth day of his polyuria but owing to operative infection the immediate effect could not be observed The slight fever he had accounts probably for the reduction of his polyuria and the rise of the Δ of his urine, which did not reach the level prevailing before puncture As soon as the fever disappeared, the polyuria reappeared with exactly the same characteristics as before—the same Δ of -2.0 as against -3.4 before the puncture and the same ability to concentrate under the influence of pituitrin and restriction of fluids

In Dog 23 a puncture polyuria was produced after the kidneys had been denervated

We come thus to the important conclusion that the experimental diabetes insipidus is not suppressed by denervation of the kidneys and that the vasomotor polyuria following denervation may be superimposed on the diabetic polyuria

(4) *Coma, Convulsions and Cachexia*—Dog 4 died on the second day in a state characterized by coma and convulsive seizures Dog 3 after an apparent recovery in which he was dull but ate normally

28 Quinby Am J Physiol 42 592, 1916, J Exper M 23 535, 1916

fell into a progressive cachexia with hypothermia and died in convulsions. It is true that Dog 4 had signs of infection in his left temporal region but this could not possibly be the cause of the immediate coma. No other finding could account for this clinical picture than the lesion of the hypothalamus just back of the pituitary stalk. In both the integrity of the pituitary was verified histologically beyond question.

Three other Dogs, 5, 23 and to a certain extent 10, in the days immediately following the operation showed the syndrome so well described by Cushing and called by him cachexia hypophyseopriva, head drooping, back arched and apathy. All these animals had polyuria, one glycosuria and one later developed the adiposogenital syndrome.

5 *The Adiposogenital Syndrome*—In Dog 3 there was an acute testicular atrophy. The spermatozoa and spermatids were almost completely absent with degenerative changes in the other cells. It is probable that this dog would have developed the adiposogenital syndrome if he had lived.

The entire syndrome developed insidiously in Dog 10. After a slight apathy for a few days he seemed perfectly normal except for his polyuria. Then he began gradually to gain weight and grow apathetic and sleepy. He gained 8 kg in three months. His testes and penis atrophied and he showed a complete indifference in the presence of a bitch in heat. The glucose tolerance could not be tested because he invariably vomited the sugar but intramuscular injection of 4 cc of adrenalin did not provoke a glycosuria. His hair which had been very rough became smooth and thin. He was also irresponsive to painful stimuli.

Dog 22 also had a testicular atrophy and gained considerable weight, most of which he lost, however, because of an acute infection, before it was necessary to sacrifice him.

DISCUSSION OF RESULTS

Perhaps the facts we have observed will throw some light on the pathogeny, still so obscure, of diabetes insipidus. We confirm the findings of Aschner, Camus and Roussy, and Houssay in showing that the polyuria is not due to injury of the pituitary gland but may be provoked with certainty by a lesion even extremely minute in the parainfundibular region of the hypothalamus. In most cases after such a lesion the polyuria is a transitory phenomenon but may be a permanent one which has all the characteristics of the diabetes insipidus observed clinically. In such cases there are also disturbances of metabolism and sexuality.

Is it possible to correlate this polyuria with those much more transitory ones induced in acute experiments by punctures or electrical stimu-

lation in various parts of the nervous system, e g , the gyrus sigmoideus (Bechterew), the cerebellar vermis (Eckhard²⁹), or the fourth ventricle (Eckhard,²⁹ Finkelnburg, Jungmann and Meyer³⁰)? We doubt it very much. These polyurias are immediate, small and very transitory. They are probably vasomotor phenomena, for at least that induced by puncture in the floor of the fourth ventricle cannot be produced after section of the splanchnic nerves (Eckhard²⁹). On the contrary, we have seen that denervation of the kidneys in dogs with experimental diabetes insipidus, not only does not check their puncture polyuria but superimposes another on it. If we exclude the old experiments of Kahler which have been contested (cauterization of the fourth ventricle with silver nitrate), there is no evidence that a permanent polyuria can be obtained by lesion in these places. We may suppose, therefore, that the polyuria provoked by puncture of the hypothalamus is unique.

The first question which arises in interpreting the results of any experimental lesion of the nervous system is: are the effects noted due to an excitation or a destruction? The short duration of most of these polyurias suggests the former possibility in our experiments. But there are all transitions between the temporary and permanent polyurias as is also the case with clinical diabetes insipidus. Furthermore, study of the lesions shows that the location of the lesion is the essential factor. When the lesion is on the border of the necessary center or is extremely minute the polyuria is transitory. It would seem that destruction of the proper cells is necessary to a permanent polyuria. Besides in these cases there were other symptoms (cachexia, adiposity, genital atrophy) which are difficult to explain by an excitation. Of course, so far as the polyuria is concerned, the destruction of a nerve center may suppress an inhibition and so act ultimately as an excitation.

The second question which arises is: are the effects produced by direct nervous activity or through the mediation of some organ? We have seen that most probably the kidney is not involved. But we must consider one hypothesis both because of its historical interest and because of the seduction it offers to the mind, namely, the hypothesis of the pituitary origin of diabetes insipidus.

From the posterior lobe of the pituitary gland may be extracted a substance which has the remarkable property of being able to reduce all polyurias and especially that of diabetes insipidus. Although pituitary extract has a transitory diuretic effect, which was the first known, its ultimate action is a long lasting oliguria particularly apparent when there is a polyuria. Here is more than necessary to offer explanation of diabetes insipidus and in fact it has been attributed both to excessive

29 Eckhard Ztschr f Biol 44 1903

30 Jungmann and Meyer Arch f exper Path u Pharmacol 73 49, 1913

secretion of the diuretic pituitary extract by the posterior lobe (Schafer) and to lack of the oliguric pituitary extract (most clinicians)

The first explanation can be rejected immediately because the diuretic effect of pituitary extract is transitory, and inconstant, being already absent on the second injection (tachyphylaxy) The second explanation requires more attention Pituitary extract even when injected slowly has in the normal animal a definite inhibitory action on the diuresis Oehme and Oehme, after careful study, concluded that it acts directly on the kidney cells (or perhaps on the ultimate ganglia) since its effect is not influenced by denervation of the kidneys and that it probably acts by storage in the cell and not by fall of concentration The effect far outlasts the vasomotor one Can it then be supposed that diabetes insipidus is due to a defect in the secretion of the posterior lobe caused by a lesion of its regulatory center in the hypothalamus? We think not The posterior lobe may be completely removed without producing a polyuria, and the same polyuria may be produced after the hypophysis has been previously removed (Camus and Roussy ¹⁸)

Pituitrin has never been proved to be discharged into the circulation The anatomic evidence, moreover, is entirely against the supposition that the posterior lobe is a secretory organ It is composed of a mass of glia cells and fibers with a very poor blood supply That the middle lobe pours a secretion into the posterior lobe and through it into the third ventricle has been apparently disproven The hyalin and colloid masses are merely degenerative products increasing with age We have therefore no evidence that pituitrin is anything else than a pharmacologically very interesting extract Oehme and Oehme, who came to the same conclusions, point further that pituitary extract inhibits the action of theocin and the effect of theocin is not increased in diabetes insipidus as it should be if a state of hypopituitarism existed

We may add to the consideration that the action of pituitary extract is on the kidney, that there is evidence that diabetes insipidus is not due to any defect in the kidney, even functional (thirst may precede the polyuria) Besides, even clinically, pituitrin is not a specific remedy for diabetes insipidus Cases have been reported in which the injection of pituitrin did not relieve the thirst and in which there was a marked retention of urea We have recently had opportunity to observe two patients in one of whom pituitrin not only did not relieve the thirst but produced an extreme dilution of the blood (fall of the blood urea nitrogen from 12 to 6 mg on the day following the injection of 2 c c pituitary extract in four doses) and in the other an extreme edema and increase of weight of 5 kg in twelve hours The first case was a giant with a metabolism —15 (P B B H, Surg No 14,377), the other a boy, aged 17, with polydactylism and other signs of degeneration (P B B H, Surg No 14,508)

What, then, is the essence of diabetes insipidus? A prolonged polyuria may theoretically be due to a primary polydipsia, a simultaneous polydipsia and polyuria, or a primary polyuria. The following causes may play a rôle: (a) polydipsia by "unjustified" thirst (psychic), (b) polydipsia by justified thirst as it can be produced experimentally as a consequence of a period of daily exaggerated intake (Veil,³¹ Regnier,³²), (c) simultaneous polydipsia and polyuria as it can be produced by a perturbation of the chemical (diabetes mellitus) or physico-chemical regulation of the body fluids, (d) polyuria due to a disturbance of some hormone regulation of the kidney, (e) polyuria due to permanent nervous or vasomotor disturbance of the kidney, (f) functional inability of the kidney to concentrate inducing a compensatory polyuria, (g) polyuria due to anatomic lesion of the kidney. Naturally, such an enumeration is purely artificial. We have no proof that these different conditions may be realized. We may immediately exclude "a" and "g" from our dogs as they are also from the definition of diabetes insipidus. Practically the discussion in later years has been concentrated on two explanations: one which considered the polyuria as a compensatory one due to the inability of the kidney (anatomically normal) to concentrate and the other which considered it an obligatory polyuria due either to a renal or to an extrarenal factor.

The teleological hypothesis of the inability of the kidney to concentrate has been vigorously criticized (Veil³¹). In 1914 Aubertin and Ambard³³ showed that the kidney in diabetes insipidus concentrates. But, nevertheless, recent authors such as Leschke,² although recognizing the value of the criticism, point out that it is impossible in diabetes insipidus to make the kidney reach a normal concentration. The argument often given that in experiments of thirst there may be a concentration of the blood proves simply that the water of the tissues had not the time to replace the water lost. It is the same concentration which may occur in severe diarrhea or sweating with the normal kidney.

We come, then, to a consideration of the possibility of an obligatory polyuria due either to renal or extrarenal factor. We bring evidence that experimental diabetes insipidus is independent of the nervous (if there be such) and vasomotor regulation of the kidneys. It persists after denervation and the denervation produces its usual effects. There is a summation of the two polyurias which shows well their independence. Although the significance of this fact is not yet clear it must be remembered also that it was possible to produce a marked puncture polyuria in a nephritic dog.

31 Veil. *Deutsch Arch f klin Med* **119** 376, 1916, *Biochem Ztschr* **91** 1918, Veil and Spiro. *Munchen med Wchnschr*, 1918, p. 1119.

32 Regnier. *Ztschr f exper path u Therap* **18** 139, 1916.

33 Aubertin and Ambard. *Bull et mém Soc med d hop de Paris*, 1914.

These negative facts lead us to the consideration of an external factor. We bring now the positive fact that the polyuria can be preceded by the thirst. It is not necessarily preceded by it. The polyuria may appear and persist without any intake of water. But at any rate the polydipsia is not consecutive to the polyuria. The only possible way to reconcile these facts is to suppose that the nervous lesion produces at once thirst and polyuria. Such a combination could only arise by a disturbance of the physico-chemical equilibrium of the organism. This hypothesis has already been defended by Veil.

Veil attributes diabetes insipidus to the inability of the tissues to hold water and he tries to give to his hypothesis a chemical basis. But the abnormalities he observed in the chemical constitution of the blood in diabetes insipidus must always be susceptible of a double interpretation. It is very difficult to decide what is cause and what is effect of the excessive loss of water. In recent works, Berglund,³⁴ and Bauer and Aschner³⁵ contested the conclusions of Veil and showed that all the differences observed in the blood between normal individuals and cases of diabetes insipidus (e. g., after injection or ingestion of sodium chlorid) could be simply explained by the excessive loss of water in the latter. They found no evidence of a change in the permeability of the vessels for salt. But the failure of the chemical proof of an extrarenal factor is not surprising if we remember that the sensitiveness of the kidney to changes in the blood far surpasses the most accurate biochemical methods (Haldane and Priestly). This is proved by the well known fact that no modification can be found in the blood after intake of water, but there is an immediate diuresis.

It is actually proven that thirst is dependent not on the state of the blood but of the tissues. Cannon³⁶ has suggested that the dehydration of the tissues causes arrest of the salivary secretion and the dryness of the throat consequently stimulates the nerve endings producing thirst. Therefore, it is interesting but probably unimportant to note also that the center which we traumatize to produce diabetes insipidus in dogs is an important gustatory center (Herrick¹⁵).

SUMMARY

The intimate mechanism of diabetes insipidus is unknown. It is provoked with certainty by a lesion of the postinfundibular region of the hypothalamus. There is evidence that such a lesion produces both polydipsia and polyuria, and the polydipsia may precede. Experimental diabetes insipidus persists after denervation of the kidney and cannot, therefore, be attributed to a disturbance of its nervous or vasomotor

³⁴ Berglund. Studier over Koksaltomsattningens, Stockholm, 1920.

³⁵ Bauer and Aschner. Wien Arch f inn Med **1** 1920.

³⁶ Cannon, W. Croonian Lecture, Proc Roy Soc Lond **90B** 283, 1918.

regulation We have no evidence of any hormone regulating the kidney, especially a pituitary hormone The kidney conserves its ability to concentrate All these facts point to an extrarenal factor as the essence of diabetes insipidus and it is certainly not a coincidence that this condition is accompanied both in clinical and experimental cases by other metabolic disturbances

PATHOGENY OF ADIPOSEGENITAL DYSTROPHY

We have given proof that it is possible to provoke the cachexia "hypopyseopriva" and the adiposogenital syndrome (together with a permanent diabetes insipidus) by puncture of the postinfundibular region of the hypothalamus without touching the pituitary The integrity of the gland and of its vascular connections was verified histologically beyond question The possibility of this has been before asserted (Aschner,⁷ Canus and Roussy^{17, 18, 20}) with strong arguments in support, but positive proof was never given because of the technic employed Besides, Aschner who in 1912 claimed that acute genital atrophy without "Fettsucht" was due to extensive lesion of the tuber cinereum seems to have abandoned the idea in 1916¹⁸ and attributed all the metabolic disturbances to lesion of the anterior lobe of the pituitary We show that not the extent but the location of the lesion is all important The lesion can be so small as to be invisible to the naked eye

The question immediately arises Cannot these symptoms nevertheless be due to the pituitary by a disturbance of its innervation? The same question was discussed in relation to diabetes insipidus We believe that it must be answered negatively here also It must not be forgotten that the only nerve fibers known to go to the pituitary come from the superior cervical ganglia (Dandy,³⁷ and Berkeley³⁸) and removal of these ganglia does not lead to any of these symptoms Furthermore, pituitary symptomatology has been built up by direct experiments on the gland where minute lesions of the hypothalamus, even contusions which later would not be discoverable with the microscope in hematoxylin and eosin sections were not and could not be taken into account The situation of these nervous centers immediately adjacent to the pituitary is such that any manipulation of the gland suffices to injure them, as is seen by the occurrence of polyuria (Crowe, Cushing and Homans²¹) Therefore, it is not surprising that even the most careful extirpations of the pituitary (Crowe, Cushing and Homans) produce either quick death or cachexia or adiposity and genital atrophy It is a strange fact that Aschner, who was the first to recognize the importance of the tuber cinereum, did not try by control experiments to determine with certainty what symptoms following

37 Dandy Am J Anat **15** 333, 1913

38 Berkeley Brain **17** 515, 1894

removal of the pituitary were due to lesion of nervous centers. In his experiments on puppies Aschner obtained in successful cases adiposogenital dwarfism. Of course he gave as arguments against a nervous lesion in these cases the absence of glycosuria and polyuria. But we know that although a hyperglycemia is probably constant, glycosuria is much less frequent after puncture in this region than polyuria. And Camus and Roussy say that polyuria is much more difficult to obtain in young animals.

Besides Aschner does not give any details concerning the immediate symptoms following operation. He had a large immediate mortality from lesion of the tuber cinereum as he himself admits (four out of six animals). In almost every macroscopic verification the infundibulum was adherent to the base of the skull and in the only microscopic verification given of one adiposogenital dwarf there was evident nerve fiber degeneration and round cell infiltration in the tuber. It is surprising that Aschner, attributing the immediate fatal symptoms and acute genital atrophy in adults to lesion of the tuber, should attribute the adiposogenital dwarfism of the few surviving animals to removal of the pituitary.

If we except naturally the arrest of growth, the syndrome observed in young animals after extensive lesion of the pituitary is identical with that seen in adults (genital atrophy, adiposity, tolerance for sugar, absence of adrenalin effect). It would be incredible that such identical syndromes should be due in the young to lesion of the pituitary and in adults to a nervous lesion. Experimental lesions in the hypothalamus of puppies are therefore highly desirable.

Quite recently Lereboullet, Mouzon and Cathala³⁹ have published a case of adiposogenital dwarfism with a suprasellar tumor and integrity of the pituitary, histologically established. The recent experiments of Smith⁴⁰ and others who succeeded in causing stunting of growth in young tadpoles by removal of the pars buccalis of the hypophysis seem conclusive until one remembers the resultant maldevelopment of the adjacent nervous tissue. It is apparently not with impunity that the normal biostatic relations of the brain may be disturbed in the developing embryo.

What, then, is the function of the pituitary? It is impossible to admit that an organ with such a highly differentiated glandular structure as the pars buccalis should not have a function at some period of life or at least at some period in the development of the vertebrate phylum. But we must admit that we have little actual knowledge of its functional significance in the adult mammal. The numerous cases published of destruction of the pituitary without symptoms point

39 Lereboullet, Mouzon and Cathala. *Rev. neurol.* 28: 154, 1921.

40 Smith. *Wistar Inst. Pub. No.* 11, 1920.

in this direction. The progressive dwindling of the pars infundibularis with cystic degeneration until in man it is hardly discoverable by aid of the microscope points in the same direction.

Have we any evidence of a hyperfunction of the pituitary? The question of acromegaly and gigantism is still unresolved. The frequency of hypertrophy or adenoma of the pituitary, as Marie was the first to point out, seems to point to a functional disturbance of the gland. But how many puzzling contradictions exist (acromegaly without any demonstrable pituitary lesion [Yamada⁴¹] etc.) It should be remembered that feeding experiments have failed to reproduce the syndrome (see however the recent work of Uhlenhuth⁴² in salamanders). These feeding experiments may have great interest for the physiology of nutrition but it is dangerous to draw from them conclusions concerning the function of endocrine organs. Besides, tissue hypertrophies may be produced by a purely nervous lesion (cheiromegaly in syringomyelia). Therefore, it is not impossible that the acromegalic syndrome may be due to an effect of the pituitary growth on the hypothalamic centers. It is very suggestive in this regard that tumors of the pineal region produce metabolic and sexual disturbances of a hypertrophic nature. The fact that these troubles must be due to pressure on neighborhood centers is well shown by the constant occurrence in the syndrome of symptoms due to various lesions of the brain and cranial nerves and in some cases by the development of the peculiar symptoms such as genital hyperplasia and adiposity in the final stages of the disease (Marburg).

As to the function of the posterior lobe the experimental evidence is unequivocal. Its removal causes no symptoms. Moreover, its structure is nonglandular. Camus and Roussy are quite justified in speaking of it as an atrophied nervous lobe. But we must reckon with the fact that from it may be extracted a very active substance, pituitrin (the origin of pituitrin in the posterior lobe and not in the pars infundibularis we accept as an established fact). It is difficult to conceive that such a substance, which is found in the pars nervosa of all vertebrates, should be without significance. Since the pars nervosa is composed almost wholly of glia, it is quite possible that this material may be extracted from the glia anywhere.

We have, on the other hand, a body of facts which show that in the hypothalamus are important nervous centers controlling manifold visceral regulations. Their anatomic connections are very imperfectly known. They are intimately connected with the olfactory and gustatory (Herrick¹⁵) systems, specialized parts of the visceral nervous system. There is some anatomic evidence also that these centers have important connections with the general visceral system, for retrograde

41 Yamada. *Mitt. a. d. Med. Fakult. d. K. Univ. zu Tokyo* 18 411, 1917.

42 Uhlenhuth. *J. Gen. Physiol.* 3 347, 1921.

changes occur in their nuclei after section of the sympathetic trunk in the neck (Huet³⁶) and after destruction of the sympathetic nucleus of the vagus (Brugsch, Dresch and Lewy¹⁶)

The physiology of the hypothalamus is also beginning to be known although the experiments have usually been acute. After lesions experimentally produced have been noted disturbance of heat regulation, dilatation of the pupil, change in the pulse and respiratory rate, glycosuria, transitory polyuria, polypnoea, etc. We believe that we bring proof also that trauma of this region may cause in addition, depending on the site and size of the lesion, permanent polyuria, adiposogenital dystrophy, cachexia and even rapid death.

The mechanism of production of the adiposity and genital regression is completely obscure. The two symptoms are in some form constantly associated, which justifies the term adiposogenital dystrophy. The fact that the adiposity is out of proportion to the adiposity of the castrate excludes the possibility of considering it as a simple consequence of the genital atrophy. Besides, the adiposity, as is well known, is only one element of a far-reaching metabolic disorder (apathy, low basal metabolism, high tolerance for sugar and epinephrin, arrest of growth in the young, etc.). Furthermore, we have seen that diabetes insipidus is probably also a metabolic disturbance.

Through what intermediary, nervous or endocrine, does the lesion of the hypothalamus produce such disorders? It is at present impossible to answer the question. It must be simply pointed out that (except naturally for the genitals) it is difficult to advocate a disturbance of any particular endocrine organ, including the hypophysis, these organs being histologically normal. The difficulty of explanation is especially acute for the genital atrophy. We have seen that the degeneration of the germinal cells may appear extremely early (Dog 3). Later (Dog 10) there is also a definite sclerosis of the interstitial gland which may account for the late regression of the secondary sex characters and the loss of libido.

Camus and Roussy, assuming that the hypothalamus controls the kidney by a nervous mechanism and pointing to the common embryologic origin of the kidney and sex glands, make the same supposition of a nervous control for the latter. But we came to the conclusion that the diabetes insipidus is not dependent on the nervous control of the kidney.

If such a nervous regulation of the sex glands exist, the nerve fibers on which it depends must leave the central nervous system very high because, so far as we know, in no case of complete transection of the spinal cord, either in man or animals, has an atrophy of the genitalia been observed. The recent work of Kuntz⁴³ may

43 Kuntz Anat Rec **17** 203, 221, 1919

point to a nervous trophic regulation of the testes. The author showed that the removal of the inferior mesenteric ganglion in dogs produces a rapid degeneration of the germinal cells of the testes with an hypertrophy of the interstitial cells. The few animals operated on were killed after about three weeks and no details are given concerning their sexual capacity. The author himself attributes the degeneration observed to vasomotor disturbance. Further research along these lines is evidently desirable.

Provisionally we are more inclined to consider the genital atrophy in connection with the invariably accompanying metabolic disorders. The extreme sensitiveness of the germinative cells to toxic influences and to deficient diet (e. g. in vitamins, Allen⁴⁴) is a well known fact, and may perhaps explain the regression of the gonads in the metabolic disorders under discussion. But we realize that such words as "metabolic" merely cover our ignorance and throw little light on the subject.

The establishing of these ideas would do much to clear away the many puzzling contradictions which continue to worry the clinicians, pituitary destruction without pituitary symptoms, pituitary symptoms with integrity of the pituitary, occurrence of diabetes insipidus and adiposogenital dystrophy in encephalitis lethargica whose nuclear election is well known, frequency of occurrence of polyglandular syndromes by lesion of the pituitary region, the paradox of regression of pituitary symptoms by roentgen-ray treatment of pituitary adenomas, treatment which must destroy what little is left of the glandular cells (Béclère⁴⁵).

All these contradictions and many others may be explained by the presence in the brain stem just above the pituitary of an important head ganglion of the visceral nervous system. We believe that the time is not far distant when the neuropathologist will no more think of omitting to examine the hypothalamus than he would the motor cortex.

CONCLUSIONS

In order to distinguish among the symptoms which are commonly ascribed both in clinical cases and in experiments on animals to the lesion or removal of the hypophysis, those which may be due to lesion of the adjacent nervous centers, systematic punctures of the hypothalamus have been made in adult dogs. The lateral route of Paulesco and Cushing has been employed, which gives a perfect exposure of the region and permits avoidance of the hypophysis with practical certainty, contrary to the transsphenoidal procedure hitherto employed for the purpose. The results of the experiments may be summarized as follows:

1. The consequences of the puncture depend strictly on the localization of the lesion and, when the localization is correct, on the extent

44 Allen. *Anat Rec* **16** 93, 1918

45 Béclère. *Paris med* **11** 97, 1921

2 A lesion, even extremely minute, of the para-infundibular region of the hypothalamus provokes with certitude (in thirteen of thirteen dogs) a polyuria which appears in the first two days. According to the extent of the lesion it varies from a transient one lasting from six to eight days to an apparently permanent polyuria.

3 In the latter case other important symptoms were present, e. g., cachexia "hypophyseopriva," genital atrophy and adiposity.

4 The permanent polyuria has all the characteristics of diabetes insipidus in man, e. g., possibility of concentration when intake of fluids is restricted, when pituitary extract is injected subcutaneously or in the presence of fever, excessive polyuric action to the administration of chlorids, absence of theobromine effect.

5 The thirst may precede the polyuria. In five cases the increase in intake preceded the output by one day, and during several subsequent days there was a marked discrepancy between the intake and output. On the other hand, the polyuria may appear and persist without intake of water, e. g., in comatose animals.

6 The experimental diabetes insipidus does not depend on a disturbance of a supposed nervous or vascular regulation of the kidney. It may be induced in animals whose kidneys have previously been denervated and when present persists after denervation of the kidneys with the same characteristics.

7 Lesion of the tuber cinereum has produced in two dogs a cachexia "hypophyseopriva" with acute genital atrophy and in two other dogs an insidiously developing adiposogenital dystrophy. The integrity of the pituitary was in each case verified histologically. The same dogs had persistent polyuria.

8 An extensive lesion of the tuber cinereum is incompatible with life. The animals either die quickly or after a period of apathy in coma and convulsions.

9 Glycosuria was an inconstant result of the lesion and seemed to depend probably on the state of nutrition of the animal.

10 Lesion, even deep, of the base of the brain outside of the para-infundibular region may produce a glycosuria but never a polyuria, the operative trauma being the same as in the previously mentioned polyuric animals. The relationship of the mamillary bodies to the polyuria is undetermined. Involved in a few cases, they were uninjured in other animals which had nevertheless polyuria and other symptoms.

11 The situation of this important nervous center and the minuteness of the lesion necessary to provoke characteristic symptoms probably explains the results of operations on the hypophysis in both young and adult animals. There is no evidence at present that the lesion acts by the intermediation of the pituitary.

OBSERVATIONS ON GLYCEMIA, GLYCURESIS, AND WATER EXCRETION IN OBESITY *

CAROL BEELER, B S, AND REGINALD FITZ, M D
ROCHESTER, MINN

Joslin¹ recently emphasized the fact that many diabetics are overweight for their age, sex and height when the diagnosis of diabetes is first made, or have a history of obesity before they develop diabetic symptoms. It has seemed of interest, on this account, to observe the results of an alimentary glucose tolerance test in a series of nondiabetic obese patients in an attempt to determine how many had an abnormally low tolerance, and could, therefore, be classified on laboratory, as well as clinical evidence, as being prediabetic.

For this purpose a group of thirty-two stout persons was studied, consisting of eight men and twenty-four women who came to the Mayo clinic for obesity or for obesity and some secondary ailment which could not definitely be related to diabetes. The urine of each patient was sugar-free on routine examination.

One of the men weighed 370 pounds, one weighed 285 pounds, one 253 pounds, and the others were at least 10 per cent overweight for their age according to the Medico-Actuarial statistics.² The heaviest woman weighed 291 pounds and several others weighed more than 225 pounds. The women, as well as the men, all weighed at least 10 per cent more than the normal for their respective ages.

The glucose tolerance test was conducted according to the plan of Hamman and Hirschman.³

On the day of the test the patient ate no breakfast. A sample of blood and urine was obtained. The patient was given 100 gm glucose in 500 c c lemonade. Samples of blood and urine were obtained thirty minutes later and one hour later. The patient was given 150 c c water. Two hours later samples of blood and urine were obtained and the patient was given 150 c c of water. Three hours later final samples of blood and urine were obtained. Thus each patient drank 800 c c of fluid during the period of study and samples of blood were taken five times.

* From the Section on Internal Medicine, The Mayo Clinic

1 Joslin, E P. The Prevention of Diabetes Mellitus, J A M A **76** 79 (Jan 8) 1921

2 Medico-Actuarial Mortality Investigation. The Association of Life Insurance Medical Directors and the Actuarial Society of America, New York, 1912

3 Hamman, L, and Hirschman, F I. Alimentary Hyperglycemia and Glycosuria as a Test for Sugar Tolerance, Arch Int Med **20** 761 (Nov) 1917

The volume of each urine specimen was recorded and each was tested for sugar by Benedict's qualitative method. The blood sugar concentration was estimated by the method of Folin and Wu⁴.

The observations on obese persons were controlled by similar tests made on ten nondiabetic patients of normal weight and on eleven mild diabetic patients. To the nondiabetic patients, however, 0.66 gm glucose for each pound of body weight was given, and to the diabetic patients a routine dose of 50 gm glucose was given instead of 100 gm. We were unable to graduate the dosage of glucose on the basis of body weight in the obese, as in the few cases tried such large doses as were necessary caused prompt nausea and vomiting. We did not feel justified in giving more than 50 gm glucose to our diabetic patients as their urines had all been made sugar-free before their sugar tolerance was tested in this fashion, and we were afraid that larger doses might be harmful.

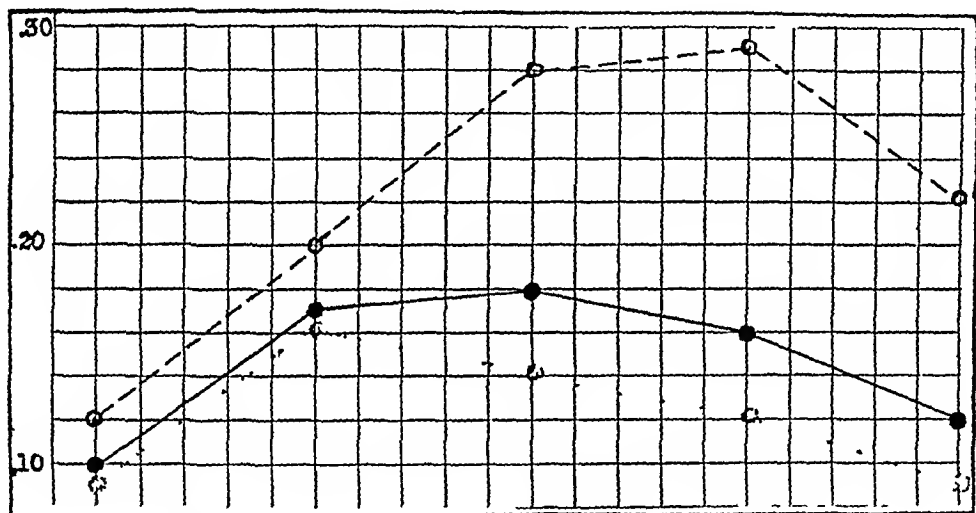


Fig 1—Blood sugar curve following the ingestion of glucose in nondiabetic patients, obese patients, and diabetic patients: dotted line, nondiabetic patients, broken line, obese patients, solid line, diabetic patients

Figure 1 shows composite curves of the blood sugar determinations in each of the three types of cases plotted by averaging all the results.

The average curve in the nondiabetic controls was not remarkable. The highest point in glycemia was reached shortly after the glucose was ingested and such hyperglycemia as occurred disappeared so promptly that the last blood sugar reading was no higher than the first.

The average curve in the diabetic patients was also typical. The blood sugar reading did not reach its maximum and did not return to its original base line as rapidly as in normal patients, and its highest point was much higher than in the control curve.

4 Folin, O., and Wu, H. System of Blood Analysis, a Simplified and Improved Method for Determination of Sugar, *J Biol Chem* 41:367, 1920

The curve of the patients with obesity is more interesting. It appears to be midway between the normal and diabetic curves, although it approaches the normal shape more closely than the diabetic. It does not suggest that most of the persons in the series according to this test were prediabetic.

When the patients are considered separately, however, certain features are noteworthy. Four patients had a fasting blood sugar above 0.15 per cent. Two of these had a normal type of sugar curve in other respects. The other two gave a reaction to glucose so typical of diabetes as more positively to suggest that they represented an early stage of the disease. The data from these two cases is shown graphically in Figure 2.

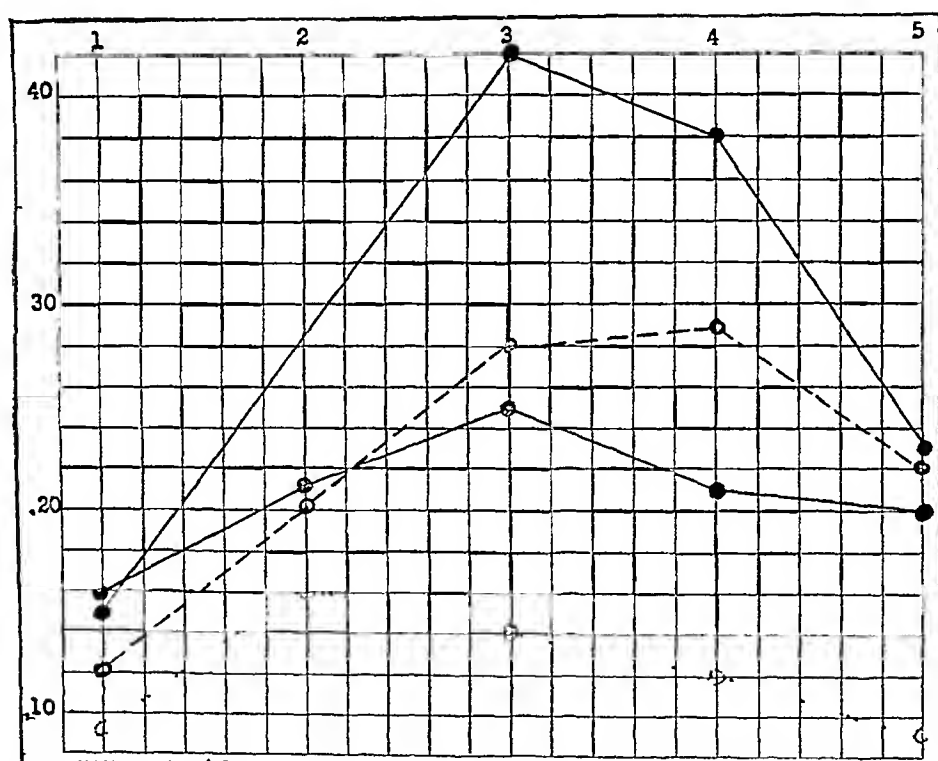


Fig. 2—Blood sugar curve in two obese patients, suggesting early diabetes

SYNOPSIS OF CASES

CASE 1—A woman, aged 32, 5 feet tall, had gradually gained from her normal weight of 120 pounds to her present weight of 164. She had no family history of diabetes or obesity. She came to the Clinic for the purpose of losing weight, and had no diabetic symptoms.

CASE 2—A man, aged 49, came to the Clinic because of palsy of cerebral origin, he had no diabetic symptoms except that he had lost 70 pounds in weight during the last fifteen months. His present weight was 196 pounds.

Six patients, while having a fasting blood sugar concentration of 0.12 per cent or less, had a persistent hyperglycemia following the ingestion of glucose and thus resembled mild diabetes in their type of

reaction The data from a marked example of this description are shown graphically in Figure 3

CASE 3—This patient was a short woman, aged 41, with incontinence due to a urethral stricture She had gained 29 pounds in the last six months owing to physical inertia and excessive eating She weighed 204 pounds

On the whole our results from blood sugar studies on obese patients with reference to the early diagnosis of diabetes mellitus were disappointing The majority of the thirty-four patients observed had no fasting hyperglycemia and had a nearly normal blood sugar curve following the ingestion of 100 gm glucose Marked deviations from normal, suggestive of diabetes, occurred in but few cases There seemed little evidence for the belief that this test as performed was of marked clinical value, except in rare instances It is probable, however, that more striking results would be obtainable if the dosage of

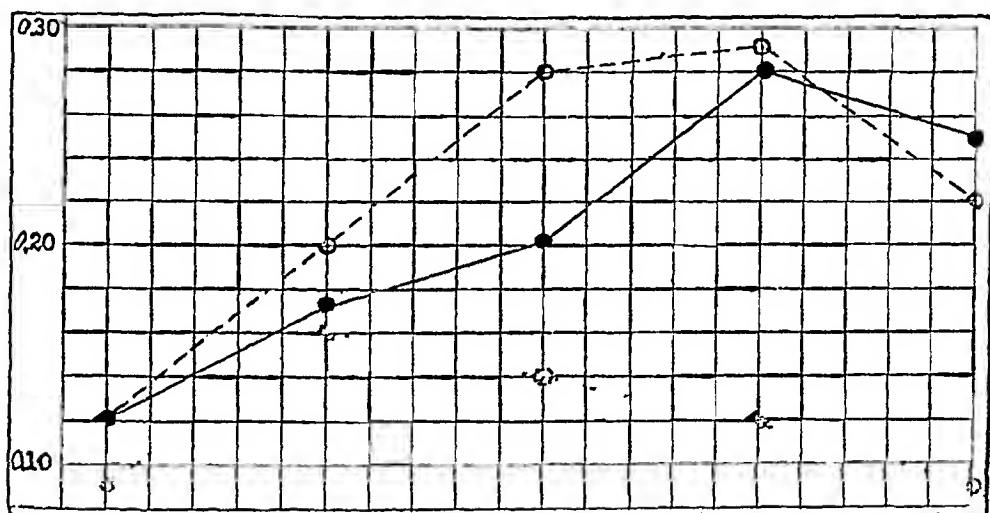


Fig 3—Blood sugar curve in one obese patient, suggesting early diabetes

glucose were made on the basis of body weight and if the sugar solution were administered intravenously according to the method of Woodyatt, Sansum and Wilder⁵

Sugar excretion was estimated quantitatively in seventeen obese, four nondiabetic, and five diabetic patients The total sugar eliminated after the sugar was ingested was determined by the method of Benedict,⁶ or of Benedict and Osterberg⁷ on the urine excreted during the period of observation

5 Woodyatt, R T, Sansum, W D, and Wilder, R M Prolonged and Accurately Timed Intravenous Injections of Sugar, *J A M A* **65** 2067 (Dec 11) 1915

6 Benedict, S P The Detection and Estimation of Glucose in Urine, *J A M A* **67** 1193 (Oct 7) 1911

7 Benedict, S P, and Osterberg, E A Method for the Determination of Sugar in Normal Urine, *J Biol Chem* **34** 195, 1918

The amount of sugar excreted by the four nondiabetic patients varied between 64 and 467 mg with an average of 233 mg. This, however, was in sharp contrast to the sugar excretion of the five diabetic patients who all excreted much larger amounts.

The excretion of sugar by the obese patients was of two distinct types. Eleven patients excreted a normal or less than the average normal amount of sugar following the ingestion of 100 gm glucose, and six patients excreted an abnormally large amount. The essential features of these observations are recorded in Table 3.

With two exceptions, all of the patients who excreted small amounts of sugar in the urine had a normal glycemia curve. The two remaining patients both had a diabetic type of glycemia so that in one the last

TABLE 1—EXCRETION OF SUGAR IN NONDIABETIC PATIENTS

Case	Sex	Age	Weight, Kg	Total Sugar Excretion, Mg	Sugar, Mg for Each Kg
1	F	35	52.2	64	1.2
2	M	42	69.5	154	1.0
3	M	56	71.7	246	3.4
4	F	18	54.5	467	8.6
Average		38	62.0	233	3.8

TABLE 2—EXCRETION OF SUGAR IN DIABETIC PATIENTS

Case	Sex	Age	Weight, Kg	Total Sugar Excretion, Mg	Sugar, Mg for Each Kg
1	M	48	76.8	2,165	28.1
2	M	40	77.2	3,920	50.7
3	M	55	91.0	5,500	60.5
4	M	47	59.1	10,300	175.0
5	F	20	43.6	12,092	275.0
Average		40	69.5	6,795	97.8

sugar reading was 0.25 per cent and in the other the first and fifth samples were within normal limits, but the three middle specimens gave readings of 0.18, 0.23 and 0.22 per cent, respectively. There was no evidence of any renal impermeability in these cases, though certain ones had symptomatic evidence of polyglandular insufficiency. These data are recorded in Part I of Table 3.

The fact that so many patients of this group excrete less than the average normal amounts of glucose after taking 100 gm pure sugar is noteworthy. It could be explained on the ground that the dose of sugar used per unit of body weight was small and that, therefore, the excretion of sugar was correspondingly small. On the other hand, the metabolism of obesity has received considerable investigation. Most authors follow von Noorden's classification of the two types of

obesity⁸ The first depends on a normal metabolism with adiposity developing through an increased food intake and a diminished energy expenditure The second depends on a true slowing of metabolism There is abundant evidence to explain the cause of the first type of case There is less evidence in favor of the second However, certain fat persons undoubtedly show a tendency to gain weight despite an intelligent regulation of diet and exercise, and others fail to lose weight when taking a low calory diet over a long period of time These facts suggest an abnormal metabolism as the true cause of the condition

TABLE 3—EXCRETION OF SUGAR IN OBESE PATIENTS

PART I						
Case	Sex	Age	Weight, Kg	Onset of Obesity	Total Sugar Excretion, Mg	Sugar, Mg for Each Kg
1	F	55	110 0	Gradual	44	0 4
2	F	39	87 4		106	1 2
3	F	32	132 1		107	0 8
4	F	21	109 0	34 kg gain in one year	138	1 3
5	F	30	89 0	15 kg gain in six months	142	1 6
6	F	54	79 5		146	1 8
7	M	45	86 4		163	1 9
8	F	62	91 0	Gradual	211	2 3
9	F	41	92 8	15 kg gain in eight months	314	3 4
10	F	59	102 0	Gradual	376	3 7
11	M	52	168 0	Gradual	428	2 6
Average		44	104 0		198	1 9
PART II						
1	F	43	91 0	15 kg gain in six months	1,019	11 2
2	M	42	91 0	Gradual	1,208	13 3
3	F	47	72 6	Gradual	2,794	38 4
4	F	26	88 5	Gradual	3,737	42 2
5	M	56	99 5	Gradual	4,950	49 5
6	F	32	74 5	Gradual	7,850	105 3
Average		40	86 0		3,593	41 7

Means⁹ recently investigated the basal metabolism in obesity, using the Benedict apparatus and analyzing his data on the basis of the DuBois formula He found no characteristic change in basal metabolism in the twelve cases which he studied, and he found that the specific dynamic action of protein was normal

Waldvogel¹⁰ attempted to study one phase of fat metabolism in obesity by injecting beta-oxybutyric acid subcutaneously and following

8 Von Noorden, C Zur Fruhdiagnose des Diabetes mellitus, Verhandl d Congr f inn Med **13** 481, 1895

9 Means, J H Studies of Basal Metabolism in Obesity and Pituitary Disease, J M Research **32** 121, 1915

10 Waldvogel Zur Pathogenese der Fettsucht, Deutsch Arch f klin Med **89** 342, 1907

its excretion in the urine and expired air His evidence is not convincing, although his conclusions are interesting He found a larger excretion of acetone in the expired air and urine of his obese patients than in control patients who had received the same dosage of acid, and, therefore, believed that in adiposity there was some disturbance in intermediary fat metabolism by which beta-oxybuturic acid was not burned to its usual completion

Wilder and Sansum¹¹ reported on the sugar tolerance of a case of myxedema and five cases of hypophyseal disease which were studied by continuous intravenous injection They found no increased tolerance in these cases by this method and concluded that the increased tolerance of such patients for sugar given by mouth is due to a retarded absorption from the bowel rather than to any anomaly of intermediate metabolism These authors obtained the same results in a few cases of obesity studied at that time

Some of our observations suggest, in spite of this evidence to the contrary, that certain obese patients may store sugar with abnormal ease or else burn it with unusual thoroughness At least this explanation would account for our findings in those patients without evidence of renal impermeability, who excreted an abnormally small amount of sugar in the urine following the ingestion of glucose, and which must have absorbed the sugar from the stomach as was shown by the temporary rise in the concentration of circulating sugar A reaction of this sort by sparing protein or fat metabolism might readily be a factor in the development of "endogenous" obesity It requires more thorough investigation

The observations on the patients who excreted abnormally large amounts of sugar following the ingestion of glucose are summarized in Part II of Table 3 All had a glycemia curve suggestive of mild diabetes It would seem justifiable to believe that obese patients who respond to a standard dose of glucose by a curve of glycemia suggestive of diabetes, and by an increased total excretion of urinary sugar, should be treated at the outset for diabetes as von Noorden¹² found that of fifteen such patients whose course he followed for a long time, five were known to develop genuine diabetes later

Several of our patients exhibited an interesting peculiarity in relation to the excretion of urine following the rapid intake of 800 c c fluid The nondiabetic controls excreted between 100 c c and 800 c c of urine under these conditions with an average excretion of 418 c c Of thirty obese patients, three excreted less than 100 c c urine in the

¹¹ Wilder, R M, and Sansum, W D *d*-Glucose Tolerance in Health and Disease, Arch Int Med 19 311 (March) 1917

¹² Von Noorden, K H Metabolism and Practical Medicine, Chicago, W T Keener Company, 1907

two hours' observation time, and only four excreted more than the average normal of 418 c c. Of the seven diabetic patients, on the other hand, only two excreted less than 300 c c urine in two hours, and the others excreted more than 500 c c, so that the average of the group was 501 c c. As a general rule, obese patients with normal blood sugar curves and without an exaggerated glycosuria excreted small volumes of urine, while those with a diabetic curve of glycemia and with an increased excretion of sugar had a relatively large volume of urine. Such oliguria in obesity has been explained by Labbé and Furet¹³ on the basis of chlorid retention and by Grafe,¹⁴ who found that interference with the water balance is not uncommon and is often dependent on interference with the thyroid mechanism for regulating intracellular water metabolism. The recent work of Larson, Weir and Rowntree¹⁵ suggests that the pituitary gland, as well, may play a part in this mechanism.

CONCLUSIONS

Obese patients show characteristic changes in sugar and water metabolism which may be of importance in subsequent treatment. Certain patients show a relatively normal curve of glycemia following the ingestion of 100 gm glucose. These patients tend to excrete small volumes of urine and small quantities of sugar. They do not appear to retain sugar because of an impermeable kidney but rather to have some disturbance in sugar and water metabolism which may be related to an endocrinopathy. It seems possible that these patients burn or store sugar with unusual rapidity, a reaction which may have a sparing influence on fat and protein metabolism and may be a factor in the development of adiposity. The effect of water retention in these patients is not known. They are probably not likely to develop subsequent diabetes, and should be treated for obesity and endocrine disease, if the existence is demonstrable.

On the other hand, another group of obese patients has a curve of glycemia following the ingestion of 100 gm glucose which resembles that of mild diabetes. These patients excrete abnormally large amounts of glucose in their urine over a measured interval of time and excrete normal amounts of urine or may even have a slight diuresis. This type of case probably represents early diabetes and should be treated accordingly.

13 Labbé, H., and Furet, L. Les troubles de l'élimination chlorurée urinaire, facteurs d'obésité, *Presse méd* **13** 809, 1905.

14 Grafe, E. Zur Pathologie und Therapie der sogenannten "konstitutionellen" Fettsucht, *Deutsch Arch f klin Med* **133** 41, 1920.

15 Larson, E. E., Weir, J. F., and Rowntree, L. G. Studies in Diabetes Insipidus, Water Balance and Water Intoxication, *Tr Assn Am Phys*, 1921.

The fasting blood sugar estimation or the glycemia curve, alone, is of little significance and is probably of less importance than the determination of glycouresis

A sugar tolerance test in obese patients which includes a blood sugar curve and quantitative measurement of sugar and fluid output affords valuable diagnostic and therapeutic information

A STUDY OF SIGNIFICANT CHEMICAL CHANGES IN THE BLOOD COINCIDENT WITH MALIGNANT TUMORS *

JOHN A KILLIAN, PH D, AND LUDWIG KAST, M D
NEW YORK

In 1897 Schopp and Moraczewski¹ concluded from their studies on urine that the development of carcinomatous growths in the human organism was characterized by a retention of nitrogen. This minus balance was attributed to an increased demand for nitrogen by the malignant tumor. More recent chemical studies on the blood in malignancy, while confirming this observation, have at the same time proffered a different interpretation of the nitrogen retention. The most comprehensive analyses of the blood in malignancy have been placed on record within the last year by Theis and Stone². Their studies included observations on the nonprotein, urea and amino-acid nitrogen, and the uric acid and sugar in a series of 189 cases, representing a wide range of type and location of malignant neoplasms. They have reported subnormal values for nonprotein and urea nitrogen, particularly in the rapidly growing tumors of the breast and uterus, and an amino-acid nitrogen slightly above normal. The uric acid was found to be normal or subnormal in all instances except two cases of melanoma. In the selection of their cases these authors state that care was taken to exclude all patients with involvement of renal function, but it is not clear on what evidence the diagnosis of renal involvement was made. On the basis of similar values for these components of the blood in pregnancy reported by other observers, an interesting analogy has been suggested by Theis and Stone between the influence of development of malignant tumors and of the embryo on the concentration of the nonprotein nitrogenous constituents of the blood of the host. The comparative decrease in the amounts of these compounds in the circulating blood has been ascribed to an increased need for nitrogen for the new growth, whether it be malignant tumor or embryo.

A number of contradictory observations have been recorded on the acid-base equilibrium of the blood in malignancy. Moore and Wilson³

* From the Laboratory of Pathological Chemistry and Department of Medicine, New York Postgraduate Medical School and Hospital, New York City

[†] Read in part before the American Gastro-enterological Society, Boston, June 7, 1921

1 Schopp and Moraczewski *Ztschr f klin Med* **33** 385, 1897

2 Theis and Stone *J Cancer Research*, **4** 349, 1919

3 Moore and Wilson *Bio-Chem J* **1** 297, 1906

report in the blood serum in malignancy an increase in basicity, which was found present in early cases and was not affected by the removal of the growth. This alleged increased basicity the authors state is not due to the disease, but rather to the advanced age of their patients. More recently, Menten,⁴ using the Michaelis method, found a normal or decreased alkalinity in only three or four out of a series of seventy patients with malignant disease, the remaining cases showing an increased alkalinity. The increased alkalinity was particularly manifest in carcinomas of the viscera but less marked in external tumors. The administration of sodium bicarbonate provoked a more rapid growth of the malignancy. Peiper,⁵ on the contrary, has observed a decrease in the alkalinity of the blood in cases of carcinoma, particularly where there is evidence of cachexia.

The concentration of sugar in the blood in malignancy probably has received more attention than any other component. Trinkler⁶ has found in all cancerous patients examined, a hyperglycemia which was more manifest in carcinomas of the internal organs than in carcinomas of the skin or mucous membranes. This increase in blood sugar, however, bears no relation to the development of cachexia. Cammidge⁷ attributes the hyperglycemia associated with carcinomas to a disturbance of the endocrine function. Of the fifty-three patients with malignant tumors examined by Benedict and Lewis,⁸ 36 per cent revealed a marked increase in blood sugar, from 0.12 to 0.16 per cent, and forty-nine per cent showed a tendency to a hyperglycemia. The blood was drawn in these cases three hours after the taking of food. The hyperglycemia was attributed by these authors to a constant demand of the growing tumor for carbohydrate. Rohdenburg, Bernhard and Krehbiel⁹ found in miscellaneous cases of malignant tumors normal blood sugar values, but after the ingestion of 100 gm glucose they obtained hyperglycemias persisting for from three to four hours. The blood sugar curves obtained bore no relation to the location of the tumor, or its nature (carcinoma or sarcoma) but closely simulated curves obtained after epinephrin injections, or in cases of hyperthyroidism. The suggestion is made by them that a probable explanation of the development of malignant neoplasms may be found in a disturbance of endocrine function. It is worthy of note, however, that glycosuria was not present in any case. A large number of the patients examined had tumors of the gastro-intestinal tract. No allowance was made by

4 Menten. *J. Cancer Research* **2** 179, 1917.

5 Peiper. *Virchow's Arch. f. path. Anat.* **116** 337, 1888.

6 Trinkler. *Centralbl. f. d. med. Wissensch.* **28** 498, 1890.

7 Cammidge. *Practitioner* **104** 131, 1920.

8 Benedict and Lewis. *Med. Rec.* **86** 650, 1914.

9 Rohdenburg, Bernhard and Krehbiel. *J. A. M. A.* **72** 1528 (May 24) 1919.

these authors for the rate of absorption of the glucose, or for a possible renal involvement. Friedenwald and Grove¹⁰ in like manner obtained characteristic blood sugar curves in carcinomatous patients after taking 100 gm glucose. The control specimens show a mild hyperglycemia, which after the ingestion of the glucose rose to 0.24 per cent in forty-five minutes and during a period of two hours did not drop below 0.20 per cent. The curve obtained in malignancies of the gastro-intestinal tract differs from those observed in carcinomas of other regions, and therefore, may be used as an aid in differential diagnosis. A hyperglycemia associated with an increased diastatic activity of the blood was frequently found by De Niord and Schreiner¹¹ in a large series of cancerous patients.

In order to determine the systemic effects of malignant neoplasms on the human organism, we began about two years ago a study of the chemical changes in the blood of cancerous patients, and this study has been pursued continuously since that time. Although no specific change in the chemical composition of the blood due to the malignancy has been observed, nevertheless, we believe the results obtained warrant the deduction of definite conclusions of practical value. Our studies comprise analyses of the blood for urea nitrogen, and in some instances for nonprotein nitrogen, uric acid, creatinin, sugar and carbon dioxide combining power. The diastatic activity of the blood has also been determined in special cases. In all of these analyses the methods recently described by Myers¹² have been followed. The specimens of blood were obtained in the morning before breakfast. The data also include the phenolsulphonephthalein excretion, the occurrence of protein and casts in the urine and the blood pressure. The principal clinical manifestations of the cases, having a possible bearing on the chemical blood findings have been noted under remarks. We have included in this series those cases in which the diagnosis of malignancy has been confirmed by operation, or by necropsy with subsequent histologic examination of the tissue. However, a few cases of carcinoma of the stomach came neither to operation nor necropsy, but in these instances there can be no question of the correctness of the diagnosis established by clinical findings, roentgenologic examination and chemical analysis of the gastric contents. Finally, we wish to emphasize the fact that the patients studied by us were not in a moribund state. On the contrary, they were patients who had presented themselves, many for the first time, at a general hospital principally for diagnosis.

10 Friedenwald and Grove. *Am. J. M. Sc.* 160 313, 1920.

11 De Niord and Schreiner. *Arch. Int. Med.* 23:484 (April) 1919.

12 Myers. *Practical Chemical Analysis of Blood*, St. Louis 1921.

CHEMICAL CHANGES IN THE BLOOD COINCIDENT WITH MALIGNANT NEOPLASMS

No	Case	Age	Blood Analyses					Phenol sulphonic phthalen in 2 Hrs	Urine		Blood Pressure		Days Under Observation	Remarks
			Uric Acid	Urea N	Creatinin	Diastatic Activity	Sugar	CO ₂ Combining Power	Protein	Cast	Systolic	Diastolic		
1	H T	50	4.6	15	1.9		0.12		—	—			14	Carcinoma of breast
2	J D	39		15	2.2				—	—			14	Carcinoma of breast
3	M D	38	2.8	15	2.1		0.12		—	—			16	Carcinoma of breast
4	M B	36	4.2	17	5.4		0.13	25	—	—			14	Carcinoma of breast
5	M M	62		46			0.14		—	—			2	Carcinoma of breast, died from postoperative acidosis
6	O S	48	3.2	12	1.6		0.13		—	—	130	48	4	Multiple sarcoma, moderate anemia
7	S B	45	4.2	13	1.9		0.12		—	—	130	68	67	Lymphosarcoma of right groin
8	A M	45		29	1.7		0.12		—	—			21	Carcinoma of tongue
9	E H	75		13	1.5		0.11		—	—			20	Carcinoma of larynx
10	W L	57		41	2.7		0.10		+	—	140	80	58	Carcinoma of lungs, necropsied
11	J F	44	5.1	16	1.7		0.18		+	—	140	90	37	Carcinoma of lungs, necropsied
12	J P	40	4.5	29	3.0		0.13		+	+	100	60		Carcinoma of esophagus, postoperative uræmic death
13	J V	67	10.1	21	6.0		0.11		—	—			3	Carcinoma of esophagus and cardia Died following operation
14	W W	63	8.0	27	3.5		0.26	52	—	—				Carcinoma of esophagus, uræmic death after gastrostomy
15	S O	50	6.8	36	2.4		0.18	52	+	—	100	79	10	Inoperable carcinoma of stomach, laparotomy
16	M R	45		11	2.1		0.16		—	—	150	80	22	Inoperable carcinoma of stomach, laparotomy
17	L B	51		12	2.0		0.11		—	—			21	Inoperable carcinoma of stomach, laparotomy
18	F T	60		13	1.8		0.12		—	—	120	70	10	Carcinoma of stomach, treatment medical
19	M O	64	3.6	14	2.0		0.12		+	+	120	90	10	Carcinoma of stomach, treatment medical
20	L T	42		14	2.1		0.11		—	—			34	Carcinoma of stomach, pyelorectomy, improved
21	T E	43		14	1.9		0.12		—	—			20	Carcinoma of greater curvature and omentum, laparotomy
22	A Y	57		15	2.0		0.11		+	—			29	Carcinoma of pylorus, improved after gastro-enterostomy
23	J M	57	4.0	15	1.8		0.12		—	—			14	Carcinoma of stomach, treatment medical
24	V O	55		15	2.2		0.12		—	—				Carcinoma of stomach, improved after gastro-enterostomy
25	A V	67		16	2.5		0.12		—	—			26	Carcinoma of stomach, treatment medical
26	A M	43		17	1.4		0.11		—	—	118	75	14	Carcinoma of stomach, laparotomy
27	M Z	68	5.4	17	2.0		0.12		—	—			13	Carcinoma of stomach, improved after gastro-enterostomy
28	F H	66	4.1	17	2.1		0.10		+	—			18	Carcinoma of stomach with metastases to liver, laparotomy
29	A D	63		17	1.9		0.09		+	+	120	70	16	Inoperable carcinoma of stomach, laparotomy
30	I K	58		11	1.8		0.11		+	—			25	Carcinoma of stomach, treatment medical
31	I S	56	4.7	19	2.6		0.09		+	—			27	Inoperable carcinoma of stomach, laparotomy
32	H M	49		19	2.5		0.16		+	—			24	Carcinoma of pylorus, improved after gastro-enterostomy
33	S M	55		22	2.3		0.11		—	—	100	60	30	Carcinoma of stomach, died following operation
34	M P	58	4.0	22	3.0		0.12		—	—	139	98	27	Inoperable carcinoma of stomach, laparotomy

35	A M	50	23	28	0 14										26	Carcinoma of stomach, treatment medical
36	A K	55	24	22	0 10										10	Carcinoma of stomach, necropsied
37	M R	40	26	23	0 11										18	Carcinoma of stomach, died after gastro-enterostomy
38	S E	58	27	26	0 10										14	Carcinoma of stomach, treatment medical
39	H J	77	30	24					+	+	+	120			60	Carcinoma of stomach, laparotomy, died
40	M S	59	30	27	0 14				+	+	+	118			75	Carcinoma of stomach, treatment medical
41	H H	42	36	28	0 14				+	+	+	100			64	Carcinoma of stomach, treatment medical
42	M H	35	37	24	0 11				—	—	—				3	Carcinoma of stomach, treatment medical
43	R T	50	40	35	0 13				+	+	+				29	Carcinoma of stomach, moderate anemia, died after operation
44	W D	37	43	20	0 13		73	Trace	++	++	++				10	Inoperable carcinoma of stomach, died following laparotomy
45	S O	60	47						+	+	+				21	Carcinoma of stomach, died after operation
46	R O	59	79	60	0 22		42		—	—	—	150			9	Inoperable carcinoma of stomach, died after laparotomy
47	J H	50	93	37	0 10				—	—	—				21	Carcinoma of stomach, died after laparotomy
48	J D	40	96	91	0 21	35	48		+	+	+	225			3	Carcinoma of stomach, pronounced anemia, arteriosclerotic fundi, 3 days later death uremic
49	C S	69	115	100	0 17	33	52		—	—	—				4	Carcinoma of stomach with metastases to liver, necropsied
50	O G	38	122	48			90	Trace				120			18	Carcinoma of transverse colon and gastro-hepatic omentum, laparotomy
51	N W	59	8	15	0 10			80	—	—	—				28	Carcinoma of descending colon, died following operation
52	W W	65	21	21	0 13			26	+	+	+	130			31	Carcinoma of cecum, died following operation
53	J S	47	75	33	0 08				—	—	—				14	Carcinoma of rectum, died following colostomy
54	E G	32	40	25	0 11				+	+	+	118			35	Carcinoma of rectum, died after radium therapy
55	J F	49	100	25	0 18				+	+	+	140			26	Carcinoma of rectum, blood specimen obtained after operation, death due to postoperative infection
56	S T	25	102	54	0 15		60		+	+	+	110			21	Carcinoma of rectum, improved after radium therapy
57	S B	63	12	19	0 13		51	62	—	—	—				10	Carcinoma of liver with metastases to stomach, laparotomy
58	A P	60	22	20	0 10				—	—	—				14	Carcinoma of liver with metastases to stomach, necropsied
59	P L	47	28	43	0 15	25	39		+	+	+	115			14	Carcinoma of liver, necropsied
60	D M	63	12	27	0 18		45	44	++	++	++	160			36	Carcinoma of pancreas, moderate anemia, necropsied
61	M W	41	13	19	0 20	22	60		++	++	++				7	Carcinoma of pancreas with metastases to liver, laparotomy
62	R Z	64	16	31	0 23	30	58		+	+	+				11	Carcinoma of head of pancreas involving duodenum, necropsied
63	J J	67	30		0 17	20			+	+	+	120			21	Carcinoma of pancreas and gallbladder, laparotomy
64	B S	60	14	18	0 13				—	—	—				42	Retroperitoneal adenocarcinoma, laparotomy
65	J F	53	21	22	0 10				++	++	++				37	Retroperitoneal adenocarcinoma, laparotomy
66	I R	70	32	30	0 13		42		—	—	—	140			14	Retroperitoneal carcinoma, laparotomy
67	M K	66	53	37	0 20		39		—	—	—	120			5	Abdominal carcinomatosis, necropsied
68	T G	69	126	58	0 17		46		++	++	++	98			16	Abdominal carcinomatosis, died in uremia 6 weeks later

CHEMICAL CHANGES IN THE BLOOD COINCIDENT WITH MALIGNANT NEOPLASMS—(Continued)

No	Case	Age	Blood Analyses						Phenol sulphone pathalein per Cent in 2 Hrs	Urine		Blood Pressure		Days Under Observation	Remarks
			Uric Acid	Urea N	Oreat- inin	Dias- tatic Activ- ity	Sugar	CO ₂ Com- bining Power		Pro- tein	Cast	Sys- tole	Dias- tole		
69	O S	54	14	11	24		0.11	65		—	—			14	Abdominal carcinomatosis, roentgen ray therapy, died
70	S L	41		28	21		0.18			—	—			29	General abdominal carcinomatosis died following laparotomy
71	P P	51		37 102	44 112		0.12	30		—	—	110	48	6	General abdominal carcinomatosis, death uremic
72	J H	40	97	68	83					++++	+++	140	80	6	General abdominal carcinomatosis, necropsied
73	J F	44		16	17		0.17		40	+	—			39	Carcinoma of left kidney with extensive metastasis, necropsied
74	J B	46	31	19	20		0.10		55	+	+			58	Sarcoma of left kidney, improved after nephrectomy
75	V G	44		54	60		0.12		Trace	++++	—	140	90	18	Hypernephroma, left hospital unimproved
76	W L	65	33	8	15		0.12	54	79	—	—	110	70	75	Carcinoma of bladder, improved after partial resection
77	J B	30	57	10 177	25 44		0.11	41	20	—	—			10	Carcinoma of bladder 3 days after subtotal cystectomy, death uremic
78	J K	50		13	17		0.10			+	—			70	Carcinoma of bladder, death due to post-operative infection
79	E P	74	37 66	14 23	22 26		0.13	50	Trace	++++	—			27	Carcinoma of bladder 5 days after resection, uremic death 21 days later
80	P L	46		15 27 42			0.11 0.13			+	—			22	Malignant papilloma of bladder 19 days after resection
81	L W	55		16	30 25		0.11			—	—			15	Before death Recurrent carcinoma of bladder, improved after roentgen ray therapy
82	S G	48	47	16 24 27	21 22 20		0.12 0.15 0.14	50	40	+	—			34	Malignant papilloma of bladder 14 days after resection
83	F H	54	57	19	20		0.10		27	+++	+			4	19 days after resection, death Carcinoma of bladder, died 4 days after excision of tumor
84	A M	50		23	28		0.13			—	—			11	Malignant papilloma of bladder, death due to postoperative infection
85	J S	40		24	20		0.09		Trace	++++	+			4	Carcinoma of bladder, died after resection
86	P V	60		39	53		0.15	40		++++	—			14	Carcinoma of bladder
87	E H	39		49	50		0.16	40		++++	—			14	After cystotomy, died 9 days later
88	J G	32	60	42	35		0.15		7	++++	—	140	80	5	Carcinoma of bladder, died following cystotomy
89	W F	66		45	36		0.12			+	—			10	Carcinoma of bladder, died following resection
90	J R	69		53	45		0.09			+	—	135 170	80 110	49	Carcinoma of bladder After cystotomy
91	F C	57	76	56	50 25 38 45		0.12 0.12 0.13 0.12	43		+	—			2	13 days after cystotomy, patient died 2 months later Carcinoma of bladder, died following resection

92	E K	45	14.9	58	8.0		0.18				12	++	—				30	Carcinoma of bladder, uremic death after resection
93	O P	59		125	6.8	24	0.18	32	++	—			—				34	Carcinoma of bladder, cystotomy 3 months previous, uremic death
94	H O	59		169	6.0		0.21	40	—	—	Trace	—	—				19	Carcinoma of bladder, died after resection
95	I L	65	9.9	13	2.3	25	0.14		+++	—		+	—				3*	Carcinoma of prostate, improved after prostatectomy
96	M W	56	5.2	13	2.5		0.15	42	+	—		+	—				11	Carcinoma of prostate, died 2 days later
97	O L	75	4.2	14	1.8		0.12		+++	—	48	+++	—				74	Carcinoma of prostate, died 6 days after prostatectomy
98	M I	75	7.5	59	4.8		0.18		+	+	33	+	+				30	Carcinoma of prostate, died in coma following prostatectomy
99	H K	67		147	1.7		0.09	40	—	—		—	—	120			11	Carcinoma of prostate, died from post-operative infection
100	J D	59		17	2.3		0.13		—	—		—	—				14	Carcinoma of prostate, radium therapy
101	W H	73		20	2.1		0.12		+	—		+	—				29	Carcinoma of prostate, improved after prostatectomy
102	W P	69		21	2.5		0.11		+	—	20	+	—				20	Carcinoma of prostate, died following perineal prostatectomy
103	S B	69		22	2.4		0.13	43	+	—		+	—				11	Carcinoma of prostate, not operated, unimproved
104	J S	62		22			0.18		+	—		+	—				9	Carcinoma of prostate, died after prostatectomy
105	J M	68		23	2.9		0.11		+	—		+	—				48	Carcinoma of prostate, improved after prostatectomy
106	P M	66		24	2.2		0.13	58	++	—	11	++	—				22	Carcinoma of prostate, died after prostatectomy
107	O T	55		45	3.2		0.14	38	+	—		+	—				43	Carcinoma of prostate, died after prostatectomy
108	O P	58		24	2.6		0.18	32	+	—		+	—				30	Carcinoma of prostate, died after prostatectomy
109	W K	69	7.5	25	3.5		0.14	41	+	—	Trace	+	—				6	Carcinoma of prostate, death uremic 4 days after operation
110	A D	19		74	2.0		0.12	24	+	—		+	—					Carcinoma of prostate, not operated, died in coma
111	S K	58	7.7	42	4.4		0.12	34	—	—		—	—				3	Carcinoma of prostate, not operated, unimproved
112	H M	69	5.6	43	4.6		0.13	28	++	—	Trace	++	—				5	Carcinoma of prostate, died after prostatectomy
113	R Y	70	7.0	41	4.1		0.13			—			—	180			12	Carcinoma of prostate, not operated, unimproved
114	P W	16		46	4.1		0.15	33	+++	—		+++	—				6	Carcinoma of prostate, not operated, After alkali therapy
115	M M	47	5.0	52	4.4		0.17	47	+	—	47	+	—				42	Carcinoma of prostate, died after prostatectomy
116	O O	38		75	6.2		0.15		—	—	57	—	—				20	Carcinoma of penis, 10 days after amputation, generally improved
117	A R	56	10.0	102	6.0		0.10	41	—	—		—	—				10	Sarcoma of uterus, improved after hysterectomy
118	A D	53	8.3	15	2.3		0.14	38	+	+	Trace	+	—	145			90	Carcinoma of cervix of uterus, died after hysterectomy
119	M W	10	10.0	31	2.9		0.10	27	+	+		+	+	180			16	Carcinoma of uterus, marked anemia 10 days later, after transfusion, not operated, died in coma
				114	19.8		0.15	70	++	++		++	++	140				Carcinoma of uterus, Wassermann ++++ After 8 days rest and dietetic restrictions After 12 days of alkali therapy, died 2 days later in uremia
				151	21.0		0.17	53	++	++		++	++	120			14	Carcinoma of cervix of uterus, on alkali and radium therapy, died 10 days after blood examination
							0.18											
							0.21											

DISCUSSION

The results of studies on chemical changes in the blood on 119 cases of various types of malignancies are presented in the table. It will be observed on inspection of these findings that in three cases only (Cases 3, 57, 95) the uric acid concentration of the blood was less than 3 mg per 100 c c. In the other cases, however, it ranged from 3.3 to 17 mg, the majority of the figures being above 5 mg. Myers¹³ and his co-workers have frequently pointed out that the uric acid concentration of normal blood is from 2 to 3 mg per 100 c c, but as the permeability of the kidney is lowered in the initial stages of renal impairment, this is first indicated in the analysis of the blood by an increase of the concentration of uric acid. This failure to excrete the uric acid is a more reliable and an earlier indication of an insufficiency of kidney function than a proteinuria or cylindruria. Later, as the involvement of renal function progresses, the urea accumulates in the blood, but, in general, there is no perceptible rise in the creatinin concentration until the urea nitrogen has doubled or more than doubled. It is evident that this order of retention of the nitrogenous waste products must depend on the comparative ease with which the kidney excretes them. Myers and Fine¹⁴ have pointed out that the kidney normally concentrates the creatinin 100 times, the urea eighty times, but the uric acid only twenty times. Hence, of these compounds the creatinin is the most readily and the uric acid the most difficultly eliminated, with the urea standing in an intermediate position. After a thorough study of tests of renal function in a large series of cases, Baumann, Hansman, Davis and Stevens¹⁵ have concluded that the rise in the uric acid concentration of the blood is the most sensitive index of a failing kidney function at our disposal. It will be noted that in many instances (Cases 1, 60, 77, 79, 83, 97) the high uric acid figures are associated with a definite decrease in the phenolsulphonephthalein excretion although normal values for urea nitrogen and creatinin are reported. This increased concentration of the uric acid in the blood must, then, be attributed to a beginning impairment of renal function.

The urea nitrogen content of normal blood has been placed by Kast and Wardell¹⁶ between 12 and 15 mg per 100 c c, and Folin¹⁷

13 Myers, Fine and Lough *Arch Int Med* **17** 570 (May) 1916, Chace and Myers *J A M A* **67** 929 (Sept 23) 1916, Myers and Fine *Chemical Composition of Blood in Health and Disease*, New York, 1915

14 Myers and Fine *J Biol Chem* **37** 239, 1919

15 Bauman, Hansman, Davis and Stevens *Arch Int Med* **24** 70 (July) 1919

16 Kast and Wardell *Arch Int Med* **22** 581 (Oct) 1918

17 Folin *J A M A* **69** 1209 (Oct 13) 1917

has stated that the maximum concentration of this component in normal blood is from 14 to 15 mg per 100 c c. It has been shown by Mosenthal and Lewis¹⁸ and by others that in the secondary disturbance of kidney function due to irritation there first develops a stage of renal hyperactivity in which a very rapid elimination of urea and an excessive output of phenolsulphonephthalein are noted. The increased excretion of the urea leads to a decreased concentration of this product in the blood. Four cases in the table (Cases 16, 30, 50, 76) show subnormal values for the urea nitrogen from 8 to 11 mg per 100 c c., and uric acid figures within normal limits. These findings are similar to many reported by Theis and Stone, but we believe the decreased urea nitrogen of the blood is due, not to any excessive demand for nitrogen by the new growth, but rather to a hyperpermeability of the kidney for the nitrogenous waste products. The fact that these low figures for urea nitrogen are associated with an increased output of phenolsulphonephthalein (from 60 to 85 per cent) lends support to our opinion. These cases apparently represent instances of renal hyperpermeability in a kidney involvement probably due to a toxemia, and analogous to conditions described by Mosenthal and Lewis.

A study of Cases 77, 79, 82, 96, 97 is particularly instructive. In these instances high figures for uric acid but normal values for urea nitrogen were obtained in the first analyses of the blood, but after an aggravation of the kidney involvement due to surgical interference, there is noted a marked increase in the urea nitrogen and a beginning retention of the creatinin. An increase in the urea nitrogen of the blood to 20 mg or more is considered by Kast and Wardell to have a pathological significance. Figures for urea nitrogen exceeding 20 are found particularly in malignancies of the viscera. In the majority of external tumors and in about 50 per cent of the cases of carcinoma of the stomach normal values for this factor have been obtained.

It is evident from the studies of Myers and Killian¹⁹ that the blood creatinin furnishes a better prognostic insight into the extent of the involvement of kidney function than either the urea of the blood or the phenolsulphonephthalein test. For normal individuals the creatinin of the blood amounts to from 1 to 2 mg per 100 c c. Figures of 3 mg or more are, with but few exceptions, accompanied by a marked urea retention, and although many patients with a creatinin concentration of 4 mg or less have shown improvement under treatment, cases with more than 4.5 or 5 mg almost invariably

18 Mosenthal and Lewis *J A M A* **67** 933 (Sept 23) 1916

19 Myers and Killian *Am J M Sc* **157** 674, 1919

terminate fatally. In the series reported here, an increase in the creatinin content of the blood is found in those cases in which the retention of urea has already reached a high level. In many instances the damage to kidney function has been sufficient to produce an accumulation of creatinin of 4 mg or more. The termination of many of these cases was typically uremic.

Since the development of malignant neoplasms in the human organism almost invariably produces a more or less severe anemia, this must be considered as a possible factor modifying the composition of the blood. While Mosenthal and Lewis noted a range of urea nitrogen in five cases of anemia, primary and secondary, from 6 to 14 mg, Tileston and Comfort's²⁰ cases of severe anemia due to hemolysis were characterized by a marked retention of nonprotein and urea nitrogen. Gettler and Lindeman²¹ recently reported in pernicious anemia a significant rise in the uric acid. The nonprotein and urea nitrogen and creatinin, though less markedly increased, were, in general, above normal. A decrease in the amount of circulating blood rather than an impairment of renal function was suggested as a cause of these abnormal values. If this were true, the increase in concentration would have been proportionately the same for all three compounds.

Bock²² has determined the total blood volume and the blood plasma volume by the vital red method in a series of thirty cases representing extremes of total corpuscle content, and wide range of total blood volumes. He has found that the blood plasma volume is very constant, forming on the average of 5.1 per cent of body weight. Variations in the total blood volume per kilogram of body weight are due to changes in the corpuscular content of the blood.

Denny²³ has utilized the changes in the oxygen capacity of the recipient's blood produced by a transfusion of a definite amount of donor's blood of known oxygen capacity, to calculate the total blood volume and the blood plasma volume in ten cases of pernicious anemia. This author has shown that although the total blood volume was decreased in all but two cases, the blood plasma volume remains essentially normal, the decrease in total volume being due to a loss of cell mass. The decrease in the enumeration of blood cells in the cases studied by Bock and Denny are more pronounced than any noted in our series. The maximum decrease in blood volume in these cases would produce at most an increase in the concentration of nonprotein

20 Tileston and Comfort. *Arch Int Med* **14** 620 (Nov) 1914.

21 Gettler and Lindeman. *Arch Int Med* **26** 453 (Oct) 1920.

22 Bock. *Arch Int Med* **27** 833 (Jan) 1921.

23 Denny. *Arch Int Med* **27** 308 (Jan) 1921.

nitrogenous constituents of but 1.2 times. It is apparent, then, that the diminished blood volume cannot account for the marked rise in the nonprotein nitrogenous constituents noted by us. Moreover, we find that all three of the nitrogenous waste products are not increased to the same extent. On the contrary, a definite order of retention has been demonstrated—the first product to be retained is the uric acid, next the urea and finally the creatinin. It is inconceivable that a diminished blood volume could produce a subnormal excretion of phenolsulphonephthalein, even before an accumulation of urea or creatinin had taken place. When the anemia secondary to the malignancy in our cases has been moderate or severe, this has been noted under remarks. There is no apparent relation between the changes observed in the composition of the blood and the anemia associated with the malignant neoplasms.

With the exception of the carcinomas of the pancreas, a hyperglycemia and an increased diastatic activity of the blood, indicating a lowered tolerance for carbohydrate, were encountered only in those cases manifesting a nitrogen retention. As this retention became more marked, the hyperglycemia, and the increased diastatic activity kept pace with it. Apparently then, the hyperglycemia noted by Benedict and Lewis, Trinkler and Cammidge and the increased diastatic activity reported by De Njord and Schreiner must be attributed, not specifically to the malignant new growth but rather to the impairment of renal function. It is well known that many cases of severe nephritis with nitrogen retention give high figures for blood sugar. Myers and Killian²⁴ have reported an increased diastatic activity of the blood and a hyperglycemia in advanced nephritis, and Williams and Humphreys²⁵ state that the level of the blood sugar in nephritis depends upon the severity of the renal insufficiency.

The tolerance for glucose in nephritis has been carefully studied by Hamman and Hirschman²⁶ who have obtained blood sugar curves after the ingestion of glucose closely resembling diabetic curves. The disturbance of renal function raises the threshold for sugar excretion so that a marked hyperglycemia may result without a glycosuria. An interesting case has been described by Bailey,²⁷ in which the blood sugar rose to 0.30 per cent before traces of sugar could be detected in the urine. The high blood sugar curves persisting after the ingestion of 100 gm glucose by cancerous patients described by Rohdenburg, Bernhard and Krehbiel and by Friedenwald and Grove, and considered by

24 Myers and Killian *J Biol Chem* **29** 179 1917

25 Williams and Humphreys *Arch Int Med* **23** 537 (May) 1919

26 Hamman and Hirschman *Arch Int Med* **20** 761 (Dec) 1917

27 Bailey *Arch Int Med* **23** 455 (May) 1919

these authors to be characteristic of malignancy, find an explanation in this disturbance of renal function. Further evidence in support of this explanation is furnished by the absence of a glycosuria in spite of a pronounced hyperglycemia (from 0.24 to 0.30 per cent.)

Four cases of carcinoma of the pancreas (Cases 60, 61, 62 and 63) have been studied by us. In two cases the diagnosis was confirmed by necropsy, and in the other two by operation. In all instances a hyperglycemia (from 0.17 to 0.23 per cent.) and a definitely increased diastatic activity (from 20 to 30 per cent.) were found. In one case there was a retention of uric acid alone, and in another an increased urea nitrogen, but the impairment of kidney function here was not sufficient to account for the hyperglycemia and increased diastatic activity. In all cases there was noted a glycosuria. This increased diastatic activity of the blood, the hyperglycemia and the glycosuria, we believe, are of some diagnostic value in malignancies involving the functions of the pancreas.

According to Van Slyke and his collaborators²⁸ normal blood plasma has a carbon dioxide binding capacity of from 53 to 77 volumes per cent. A study of the figures in the table for the carbon dioxide combining power of the blood plasma reveals a normal or slightly decreased alkali reserve of the blood in the cases of malignancy without a retention of nitrogen. We have found no evidence of an increased basicity reported by Moore and Wilson, and Menten. In Case 49 a carbon dioxide combining power of 90 was found, with a severe retention of urea and creatinin. Here, however, a diagnosis of gastric ulcer had originally been made and huge doses of sodium bicarbonate had been administered by mouth daily for a period of fourteen days previous to admission to the hospital. This alkali therapy undoubtedly was responsible for the abnormal carbon dioxide combining power of the blood. A definite decrease in the reserve alkali of the blood plasma indicating a moderate or severe acidosis is found in many cases showing nitrogen retention. A ketosis, however, was uniformly absent. There appears to be a causal relation between the severity of the kidney impairment and the acidosis noted and no doubt, the mechanism of production of this acidosis is analogous to that causing the acidosis in nephritis. Chace and Myers²⁹ have found in cases of severe nephritis an associated acidosis, sufficient in many instances to be the actual cause of death.

These observed changes in the blood cannot be explained by a nephritis independent of the malignancy, to be expected in patients of advanced age. The degree of renal impairment bears no relation

²⁸ Stillman, Van Slyke, Cullen and Fitz. *J. Biol. Chem.* **30** 405, 1917.

²⁹ Chace and Myers. *J. A. M. A.* **74** 641 (March 6) 1920.

to the age of the patients. In fact, the most pronounced retention of urea nitrogen and of creatinin observed was in a case of a woman, aged 25 years. The systolic and diastolic blood pressures are in general normal or subnormal. A hypertension was noted in but one case, where there was other evidence of general arteriosclerosis. An examination of the ocular fundi revealed normal pictures in all cases with the single exception of this arteriosclerotic (Case 48). Although proteinuria was frequently encountered, the amount of protein excreted in the urine was not a reliable index of the kidney impairment. Casts were rarely seen.

The accumulation of these nitrogenous waste products cannot be ascribed to an increased metabolism produced by the growth of the tumor within the organism, inasmuch as the removal of the neoplasm does not decrease the concentration of these nitrogenous substances. On the contrary in many instances the nitrogen retention after the operation greatly exceeds the preoperative findings.

Turning to the more practical side of this question, it is obvious that since malignant tumors in the human organism in many instances entail a secondary impairment of renal function, an examination of the kidney activity should be made as a preoperative measure. It has been our experience that frequently anesthesia and surgical interference have provoked an acute exacerbation of the renal involvement with a fatal termination. The aggravation of the kidney damage after operation is not dependent on a postoperative infection, for the effect of the surgical procedure upon the renal function appears too rapidly after operation to be due to infection. Furthermore, in three cases death was due to postoperative infection, but in two of these cases following operation there was no increase in nitrogen retention. In the third case the specimen of blood examined was obtained ten days after operation and the retention of nitrogen found may be due to a kidney involvement secondary to *B. welchii* infection. It is obvious that a chemical examination of the blood will afford the surgeon more accurate and reliable information concerning the functional capacity of the kidneys than a urinalysis. Squier and Myers³⁰ have emphasized the preoperative prognostic value of the blood urea in surgery on the prostate, and we believe their conclusions are tenable also in malignancies. Patients showing urea nitrogen figures under 20 mg per 100 c.c. may be regarded as good operative risks so far as the kidney activity is concerned, but when the urea nitrogen rises to 20 or 30 mg the patient should be operated on with great caution, and a retention to 30 mg or more indicates a poor operative risk. However, it is our opinion that patients showing an increase in the uric acid only with a

30 Squier and Myers. J Urol 2 1, 1918

subnormal phenolsulphonephthalein excretion, as Cases 77, 79, 82, 83, should not be operated on except in extreme emergency, until preliminary treatment has brought the kidney function up to normal standards. Since Killian³¹ and Short³² have reported a decrease in the alkali reserve of the blood after general anesthesia and surgical procedures, a preoperative examination of the carbon dioxide combining power of the blood in these cases, particularly where a nitrogen retention has been found, will indicate to the surgeon the necessity of guarding against a postoperative acidosis.

SUMMARY

Of the 119 cases of malignancy examined, about 80 per cent show a definite increase in the uric acid concentration of the blood, and about 60 per cent an increase of the urea nitrogen and creatinin, indicating a more or less severe impairment of kidney function. The appearance and progress of this renal insufficiency follows the order of retention of the nitrogenous waste products characteristic of nephritis of the interstitial type. This impairment of renal function was found invariably in general abdominal carcinomatosis, in about 90 per cent of cases of carcinoma of the bladder, prostate, uterus and rectum, in about 50 per cent of cases of carcinomas of the stomach, and rarely in external tumors. In all cases of nonmalignant tumors no such disturbance of kidney function has been noted. The accumulation of the nitrogenous waste products in the blood was paralleled by a decrease in phenolsulphonephthalein excretion, but many of the other signs indicative of nephritis, e. g., hypertension and changes in the ocular fundi, were lacking. The extent of the renal insufficiency was independent of the age of the patients and the associated anemia. Disturbances of carbohydrate tolerance were found to be dependent on the kidney involvement rather than on the malignancy itself. An acidosis was encountered in many instances, particularly in cases showing nitrogen retention. A pre-operative chemical examination of the blood is of great prognostic value in malignancy, since it serves as an excellent index of renal function and also of any acidosis.

31 Killian *Proc Soc Exper Biol and Med* **15** 17, 1917

32 Short *J Biol Chem* **41** 503, 1920

A STUDY OF THE UREA CONCENTRATION TEST FOR KIDNEY FUNCTION *

I M RABINOWITCH, M D

MONTREAL

Advances made in recent years in laboratory methods for the study of kidney function have extended our interests beyond the observation of structural changes. The finding of albumin, casts, blood or pus in the urine, is in the great majority of cases definite evidence of structural changes in the kidney. Newer laboratory methods have, however, taught us that anatomic and functional integrity of the kidneys are not synonymous terms. Serious functional changes may be found clinically, and at necropsy no commensurate degree of tissue destruction may be noted. Extensive tissue destruction may be found at necropsy, and with our present methods of determining functional activity, no marked impairment may be found clinically. Much is still unknown regarding the physiology of the kidney. For this reason any new method devised as a means of determining kidney function is welcomed by the clinician.

In February, 1920, MacLean and de Wesselow¹ reported a method for determining kidney function, which they called the "new urea concentration test." These authors found "that when a large dose of urea was administered to a patient with defective kidneys, the patient was incapable of excreting urine with a high urea concentration." The test appeared to be a quantitative one. From their findings they concluded that in their experience it was of more value in indicating deficiency of kidney function than other tests then (1920) in use.

Details of the test as described by its authors are as follows: "The patient empties his bladder and immediately afterward takes 15 gm urea dissolved in 100 c c water. One hour after taking the urea, he passes water. This sample is kept for estimation of urea percentage. Two hours after administration of the urea he again passes water. This sample is also kept for estimation of urea percentage."

The urea was estimated by the hypobromite method. Any concentration over 2 per cent was taken as an index of fair efficiency; below 2 per cent was considered unsatisfactory, and the lower the concentration the more serious the lesion. The value of the method

* From the Department of Metabolism of The Montreal General Hospital

1 MacLean, H., and de Wesselow, O. L. V. On the Testing of Renal Efficiency, with Observations on the Urea Coefficient, Brit J Exper Path. 1 53 (Feb) 1920

as a quantitative test was emphasized, and they criticized the "urea coefficient test" (MacLean Index) which they regarded as a qualitative test only and in the presence of a normal blood urea it "is only a waste of time and labor to utilize a complicated mathematical formula, when the same information can be obtained from the urea concentration alone"

While attempting to compare this method with other methods employed in the routine of examination of kidney function in the Montreal General Hospital, an opportunity was afforded, at the same time, of reviewing the work done on kidney function during the last year. Practically every well known method has been given consideration. Eventually, a routine has been adopted which seems, in our experience, to be practical and to give the desired information. Up to the present, the minimum investigation carried out in all cases admitted to the wards, which were found to have an albuminuria, consisted of the estimation of the blood urea, blood creatinin, and of the percentage excretion of phenolsulphonephthalein in two hours.

Table 1 shows the general routine followed.

TABLE 1—ROUTINE ADOPTED FOR ESTIMATING KIDNEY FUNCTION

Clinical Condition	Examination									
	NPN	BU	BCr	UrAc	NCI	PST	RTM	NU	AUT	DIO
Hypertension	+	+	+	+	+	+	+			
Suspected early nephritis	+	+	+	+	+	+	+			
Acute nephritis		+	+		+	+		+	+	+
All edemas		+	+		+	+		+	+	
Surgical kidneys (preoperative)		+	+			+		+	+	
Prostatic enlargement (preoperative)	+	+	+			+	+	+	+	
Cardiac cases (for diagnosis)	+	+	+		+	+	+	+	+	
Cardiac cases (for progress)		+			+	+		+		+

Explanation of abbreviations: NPN, nonprotein nitrogen of the blood, BU, blood urea, BCr, blood creatinin, UrAc, blood uric acid, NCI, Actual, calculated and threshold of blood chlorides, PST, phenolsulphonephthalein, RM, Mosenthal renal test meal, NU, night urine, AUT, urea concentration test (MacLean and de Wesselow), DIO, daily intake and output of fluids.

Judging from our experience, with this routine laboratory procedure, we cannot help but conclude that there is no one single test for kidney function, which, employed to the exclusion of all others, has not its limited sphere of usefulness. It may reveal a fraction of the cause of the clinical symptoms, and a very valuable one, but at its best, it is only a laboratory aid, and must be correlated with the clinical picture in order to reach a final diagnosis, which latter has justly been called "a complex clinical judgment." The reason for this seems quite obvious. The study of a pathologic reaction is the study of a disturbed physiologic reaction, and the exact physiology of the kidney is still obscure.

A careful study was made of this new "urea concentration test" to determine its value as compared with the routine methods now in use. No choice was made of the cases clinically. These were studied as they were admitted to the wards, and included marked cardiac, cardiorenal, marked renal cases, and those cases admitted for other clinical pictures but which were also found to have an albuminuria.

Table 2 presents a list of the first fifty cases studied. Since the same conclusion could be drawn from the study of subsequent analyses, further tabulation seemed unessential.

In all the determinations of the urea in the blood and urine, the very accurate method of Van Slyke was employed.

The minimum information obtained in each case consisted of the percentage of blood urea, blood creatinin, percentage of phenolsulphone-phthalein excretion in two hours, and the urea concentration test. Wherever it was clinically possible a complete Mosenthal renal test meal was done. In nearly every case the character of the night urine was studied. This was done by allowing the patient a full hospital diet. No food nor fluids were allowed after the evening meal. At 8 p. m. sharp the patient voided urine. This specimen was discarded. All subsequent specimens were collected until 8 a. m. the following day. This twelve hour specimen was examined for volume and concentration of nitrogen. We have termed this the "nocturnal urine test." No originality is claimed for this test. Mosenthal in his original description² of his modification of the Hedinger and Schlayer meal pointed out that it was unnecessary to adhere exactly to the details of the meal when no balance determination was required, and Christian, in describing his modification of the meal,³ again emphasized this point.

In each case the clinical picture was studied as completely as possible, and an attempt was made to correlate this with the functional findings.

In interpreting our observations, a few theoretic considerations seem essential. Since the test centers about the excretion of urea a little consideration of the physiology of this product is necessary.

Urea is the chief nonprotein nitrogenous element found in the urine. On an ordinary diet, in a normal person, its nitrogen content may be as much as from 80 to 90 per cent of the total nitrogen excreted during the day. From this consideration alone it appears that this new test might be similar to one already in use, and duplication of work must be avoided in a large general hospital with a large routine. In describing

² Mosenthal, H. O. Renal Function as Measured by the Elimination of Fluids, Salt and Nitrogen, and the Specific Gravity of the Urine, *Arch. Int. Med.* **16** 733 (Nov.) 1915.

³ Christian, H. A. The Use of Tests of Renal Function in Cases of Nephritis, *J. Urol.* **1** 319, 1917.

TABLE 2—CASES ARRANGED ACCORDING TO THEIR DEGREE OF CONCENTRATION OF BLOOD UREA

No	Hos pital No	Blood, Mg per 100 C c			Urine					Clinical Remarks
		Urea N Before	Urea N After	Creat- inin	Urea Con- centration, per Cent		Night Urine		Phenol sulphone phthalein per Cent in 2 Hours	
					1st Hour	2d Hour	C c	Per Cent N		
1	1066	11	17	1 20	1 74	2 02	580	0 802	58	Chronic interstitial nephritis
2	1411	12	25	1 75	1 76	2 13	510	0 696	55	Chronic interstitial nephritis
3	1254	13	30	1 59	2 28	2 04	430	0 896	41	Cardiorenal, edema
4	1573	14	30	1 10	2 59		310	1 260	32	Chronic nephritis, pregnancy
5	1595	15	25	1 75	1 92	1 86	720	0 644	60	Rheumatoid arthri- tis, albuminuria
6	1498	16	32	1 40	1 20	1 26	600	0 840		Perineal fistula, albu- minuria
7	1557	16	33	1 20	1 32	0 96	500	0 560	45	Acute nephritis
8	1896	16	30	1 59	3 0	3 00	220	2 100	55	Acute nephritis, ery- sipelas
9	1783	17	26	1 40	1 63	1 49	540	1 17	38	Prostatic hyper- trophy, chronic ne- phritis
10	X	17	26	2 0	0 66	0 72	560	0 410	32	Hypertension, chr nephritis
11	1540	18	35	1 59	1 80	1 86	580	0 656	42	Prostatic hyper- trophy, chronic ne- phritis
12	1737	20	35	1 40	2 56	2 91	125	2 20	58	Sarcoma, albumi- nuria
13	1045	21	28	1 61	1 36	2 43	320	1 40	62	Malaria, albuminuria
14	1605	21	32	1 91	1 80	1 80	580	0 621	21	Puerperal septicemia
15	1647	21	30	1 80	1 70	1 78	600	0 105	52	Chronic interstitial nephritis
16	1680	21	35	1 30	2 42		425	0 921	50	Early interstitial ne- phritis
17	1772	21	26	1 00	2 59	2 76	220	1 00	76	Turpentine poison- ing, chronic ne- phritis
18	1836	21	30	1 90	1 72		500	0 920	44	Chronic interstitial nephritis
19	X	21	38	1 75	2 34	2 76	570	0 760		Acute pancreatitis, albuminuria
20	X	23		1 40	4 04	3 36	162	1 360	46	Acute nephritis em- pyema
21	2015	21	39	1 03	2 36		250	1 26		Cardiac, albumi- nuria
22	2015	19		1 57	2 07	2 28	250	1 70	45	Carcinoma, chronic nephritis
23	2014	16	26	2 10	0 73	1 68	450	0 682	42	Acute nephritis
24	2089	19	32	1 15	2 46	2 73	260	1 96	62	Chronic tuberculosis, albuminuria
25	1452	24	35	1 80	1 87	1 92	690	0 346	31	Prostatic hyper- trophy, chronic ne- phritis
26	1505	25		1 50	1 44	1 51	600	0 300	22	Cardiac, passive con- gestion
27	1702	25	36	1 75	3 00	3 00	430	0 840	58	Pericarditis, albumi- nuria
28	1686	26	46		3 00	3 00	235	1 730	38	Passive congestion
29	5535	28		1 90	1 38		760	0 462	30	Prostatic hyper- trophy, chronic ne- phritis
30	1921	28	42	2 00	2 13	1 44	540	0 620	52	Acute nephritis
31	1540	28	51	1 50	2 22	2 36	520	0 640	41	Prostatic hyper- trophy, chronic ne- phritis
32	1893	31	43	1 40	1 90	1 90	560	0 421	27	Advanced cardiac chronic nephritis
33	1788	32	49	1 75	1 56	1 56	700	0 780	Trace	Advanced cardiac, chronic nephritis
34	1744	32	42	1 80	1 44	1 40	520	0 588	47	Chronic nephritis
35	1172	32	43	1 61	1 92	2 64	400	1 35	55	Myxedema, albumi- nuria
36	1577	29	41	1 75	2 22	1 62	425	0 09	45	Fracture of femur, albuminuria
37	1690	32	35	2 00	2 79	2 79	540	0 840	50	Pneumonia, acute ne- phritis

TABLE 2—CASES ARRANGED ACCORDING TO THEIR DEGREE OF CONCENTRATION OF BLOOD UREA—(Continued)

No	Hos- pital No	Blood, Mg per 100 C c			Urine					Clinical Remarks
		Urea N Before	Urea N After	Creat- inin	Urea Con- centration, per Cent		Night Urine		Phenol sulphone phthalein per Cent in 2 Hours	
					1st Hour	2d Hour	C c	Per Cent N		
38	1300	32	47	1 70	1 26	1 64	600	0 360	26	Prostatic arterio- sclerosis
39	1922	34	45	2 40	1 92	2 25	640	0 561	54	Prostatic, chronic ne- phritis
40	1874	34	46	2 30	1 35	1 32	540	0 520	15	Hypertension, arte- riosclerosis
41	1749	35	43	1 75	1 35	0 95	480	1 130	50	Chronic interstitial nephritis
42	1639	35	42	1 66	2 25	2 64	560	1 103		Prostatic, chronic nephritis
43	1676	34	45	2 20	2 10	2 00	500	0 780	55	Prostatic, chronic nephritis
44	1429	35	46		2 30	2 42	600	0 320	41	Chronic lead poison- ing, chronic ne- phritis
45	1451	31		2 13	2 66	2 43	600	0 620	39	Chronic interstitial nephritis
46	1427	49		1 66	3 19	3 30	580	0 820	36	Hernia chronic ne- phritis
47	2011	49	58	1 66	2 76	2 91	640	0 820	45	Chronic interstitial nephritis
48	2103	35		1 76	1 50	1 74	660	0 320	37	Advanced cardiorenal
49	1916	60	67	3 00	1 78	1 80		0 860	Trace	Advanced chronic ne- phritis
50	1763	133	140	8 75	1 20	0 84	760	0 220	Trace	Advanced chronic uremia

the renal test meal Mosenthal has shown² that the normal kidney is able to excrete urine with a nitrogen concentration of over 1 per cent. Since normally from 80 to 90 per cent of the total nitrogen is excreted as urea, to state that any concentration of over 2 per cent urea is an index of fair efficiency, seems to be a restatement of Mosenthal's finding that a concentration of over 1 per cent nitrogen is normal. This becomes quite apparent on an analysis of the chemical

formula for urea, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{C} = \text{O} \\ \diagdown \\ \text{NH}_2 \end{array}$. It will be observed that for each sixty

parts of urea there are twenty-eight parts of nitrogen. That is, 2 14 times the nitrogen equals the urea. That this actually is the case seems well borne out on analysis of Table 3. Every case showing a low urea concentration test, also showed a low concentration of nitrogen (under 1 per cent) in the night urine. Incidentally, nearly every case showing either a low nitrogen concentration or low urea concentration also showed a polyuria (over 400 c c). This nocturnal polyuria has long been known clinically as evidence of impairment of kidney function. Although in the great majority of cases the administration of urea by mouth did not inconvenience the patients, occasionally vomiting or nausea did follow. This must necessarily be avoided in acutely ill cases. The determination of the character

of the night urine does not in any way whatever interfere with the patient, and from its consideration so far its results parallel those of "the urea concentration test"

Another factor to be considered is the rate of absorption of urea from the alimentary tract. In any test involving the administration of substances by mouth this becomes an important factor. Allen⁴ has pointed out the varying results obtained when equal amounts of sugar are given by mouth, subcutaneously or intravenously. Recently in a study of nitrogen elimination McEllroy and Pollock⁵ have shown that the rate of elimination of nitrogen is an index of the rate of absorption. That this factor of absorption plays an important part in the results obtained in the "urea concentration test" seems apparent, from a study of Table 3 and after certain physiologic phenomena are considered. Marshall and David⁶ have shown that urea is evenly distributed throughout the tissues of the body in the same concentration as in the blood. It has been assumed⁷ that for each kilogram of body weight there are about 700 c.c. of fluid. This has been later shown to be remarkably accurate.⁸ If, then, we accept this figure as correct, the administration of 15 gm. urea (7 gm. nitrogen) by mouth to a patient weighing about 60 kg. (taking for granted that urea is readily soluble, and undergoes no chemical alteration in the body, and is evenly distributed) should raise the concentration of urea nitrogen in the blood about 16 mg. per 100 c.c. Table 3 shows the effect of the oral administration of 15 gm. urea on the blood.

TABLE 3—EFFECT ON BLOOD OF INGESTION OF 15 GM. UREA

Time of Test	Blood Urea N, Mg. per 100 C.c.
Before administering urea	19.2
10 minutes after administering urea	22.1
20 minutes after administering urea	23.6
30 minutes after administering urea	22.1
40 minutes after administering urea	20.2
50 minutes after administering urea	21.0

At the end of this fifty-minute period the patient voided 160 c.c. urine with a urea concentration of 31 per cent. That is, 4.96 gm. urea were excreted. Reconsidering the previous mathematical observations, some disturbance in the absorption seems to have

⁴ Allen, F. M. *Glycosuria and Diabetes*, Harvard Univ. Press, 1913.

⁵ McEllroy, W. S., and Pollock, H. O. On the Rate of Nitrogen Elimination, *J. Biol. Chem.* **46** 475 (May) 1921.

⁶ Marshall, E. K., and Davis, D. M. *J. Biol. Chem.* **18** 53, 1914.

⁷ Palmer, W. W., and Van Slyke, D. D. Studies of Acidosis, *J. Biol. Chem.* **32** 500, 1917.

⁸ Reismann, S. P., and Reimann, H. A. Blood Bicarbonate Levels Following Administration of Sodium Bicarbonate, *J. Biol. Chem.* **46** 493 (May) 1921.

occurred. At no time during the above period did the concentration of the blood nitrogen reach the expected level. Since only 4.96 gm urea were excreted, one cannot readily assume that the failure of the rise in the blood urea is due to rapid elimination. There are still 10.04 gm to be accounted for. An analysis Table 1, which shows the urea nitrogen concentration in the blood one hour after giving the urea in each case, shows a marked irregularity in the increase. Since all the cases recorded are those with nephritis of varying degrees, a much greater increase should occur in those cases of the more advanced type. Such an increase is markedly conspicuous by its absence. For example, Case 50, which is clinically a case of kidney disease, markedly advanced, with retinal changes, and in a pre-uremic state, shows no greater increase after administration of urea than Case 5 whose only evidence of nephritis is the presence of albumin and casts in the urine. Lack of absorption of the urea in this advanced case certainly seems to have occurred.

Certain other factors apparently come into play in the excretion of urea. Ambard and Weil have shown, and this has been further exemplified by McLean,⁹ that at least in the normal case a relation exists between the concentration of urea in the blood and its rate of excretion. Further studies by Austin, Stillman, and Van Slyke¹⁰ although with somewhat different results show again that some relation exists.

Disregarding all these previous considerations it does not even then seem possible that this test unlike all others has a general application. The little knowledge that we have regarding kidney function has shown that disturbances which occur in nephritis show many variations. One type may show faulty excretion of water, another of salt, another of nitrogen, and another of the various dyes. Some show a combined picture of all these disturbances. Animal experimentation has repeatedly shown this. Folin and Denis¹¹ have had the rare opportunity of studying the kidney function in the human being following an operation of a double ureterostomy. They showed that excretion of urine by the kidney can be subdivided into a number of more or less independent excretions. In moderate hydronephrosis complicated by pyelitis the damage is not uniform. Phosphorus excretion does not correspond to chloride excretion. Creatinin and uric

9 McLean, F. C. The Numerical Laws Governing the Rate of Excretion of Urea in Man, *J. Exper. M.* **22** 212, 1915.

10 Austin, J. H., Stillman, E., and Van Slyke, D. D. Factors Governing the Excretion Rate of Urea, *J. Biol. Chem.* **46** 91, 1921.

11 Folin, O., and Denis, W. Some Observations on the Selective Activity of the Human Kidney, *J. Biol. Chem.* **22** 321, 1915.

acid do not vary together Recently Oliver¹² has shown that urea is found in a greater concentration in the proximal convoluted tubules, much higher than that of the blood, or of any other part of the tubules Therefore, damage to this part of the kidney would naturally alter the excretion of urea more than would damage to any other part of the kidney Again, many extrarenal factors, especially disturbances of the circulation, would effect the type of urinary excretion

These observations are borne out by analysis of the cases studied The following case reports exemplify the possible selective activity of the kidney

REPORTS OF CASES

CASE 1 (Hosp No 2014-21) —Female, aged 40, admitted to the Montreal General Hospital with a clinical diagnosis of acute nephritis and general anasarca

Urmalysis—Albumin, 4 plus, blood and epithelial casts, red blood cells

The patient was placed on the routine orders of restricted fluids, salt free diet, purgation, hot packs, and improvement was noted daily

TABLE 4—CLINICAL COURSE OF CASE 1

Date	Urine					Blood		Urea Concen- tration per Cent		Phenol sul- phone- phthal- ein per Cent in 2 Hours
	C c	Albumin Gm per Liter	NaCl Gm per Day	Night Urine		Urea N, Gm per 100 C c	Creat- inin, Gm per 100 C c	1st Hour	2d Hour	
				C c	Per Cent N Conc					
May 13	1,160	3 0	3 4			16	2 1	1 73	1 68	42
May 14	1,160	2 3	4 0	500	0 540					
May 15	680	2 1	7 0	400	0 708	16	2 0	1 20	1 23	50
May 16	950	2 0	5 7	400	0 920	21	1 4	1 20	1 56	56
May 17	975	2 0	4 0	375	0 588					
May 18	500	1 7	2 5	250	0 763	16	1 2	1 20	1 32	62
May 19	980	0 3		450	0 728					

There was an average of three diarrheal stools per day

In this case it will be noted that although the "urea concentration test" showed impairment in function, the nocturnal urine test gave the same information Neither the "urea" nor the nocturnal urine test showed any quantitative change indicating the progress towards recovery Recovery was shown by the elimination of fluids greater than intake, a constant excretion of salt with no intake of salt, an increase in the two-hour excretion of phenolsulphonephthalein, a gradual loss of edema, and a general improvement clinically The patient on discharge from the hospital still showed a low concentration of urea in the "urea concentration test," and also a low nitrogen concentration in the nocturnal urine test

¹² Oliver, J Mechanism of Urea Excretion, J Exper M **32** 177 (Feb) 1921

CASE 2 (Hosp No 1896-21) —Male, aged 42, admitted to the Montreal General Hospital with a clinical diagnosis of acute nephritis

The patient was too ill to carry out complete analyses. From the data obtained, however, it will be noted that there was no apparent disturbance in the nitrogen elimination, neither in the night urine nor in the "urea concentration test." At necropsy the clinical diagnosis of acute nephritis was corroborated.

TABLE 5

Date	Blood		Urine				
	Urea N, Mg per 100 C e	Creatinin, Mg per 100 O e	Night Urine		Urea Concentration, per Cent		Phenol- sulphone- phthalein, per Cent in 2 Hours
			C e	Per Cent Nitrogen Concen- tration	1st Hour	2d Hour	
May 7	18	1.59			3.1	3.2	43
May 9					3.0	3.0	16
May 10	16	1.59	220	2.1	3.2	3.1	10
May 13					3.4	3.3	
May 16	21	1.42	150	2.0	3.1	3.0	

This patient died

CONCLUSIONS

1 There is no one single test for kidney function, which, in our experience, could be used for the purpose of renal diagnosis, to the exclusion of all others.

2 Various types of nephritis show varied responses to each test.

3 The information obtained from the simple nocturnal urine test parallels, in our experience, that obtained from the urea concentration test.

4 Both the "urea concentration test" and nocturnal urine test gave only qualitative information.

5 To properly interpret the results of any test, a correlation with the clinical picture is of paramount importance.

I am very much indebted to the chiefs of the various services in the Montreal General Hospital, without whose cooperation this work could not have been done.

THE ACTION OF THE NITRITES ON THE CORONARY CIRCULATION *

FRED M SMITH, M D

CHICAGO

The nitrites have been employed in the treatment of angina pectoris since the time of Lauder Brunton, and it is well known that in many instances the pain subsides soon after the administration of a pearl of amyl nitrite or a pellet of nitroglycerin. The beneficial action of the nitrites has been considered by some to be due to the fall of blood pressure, and by others it has been held that these drugs dilate the coronary arteries and thus improve the coronary circulation thereby affording relief from the cardiac distress. Experimental observations designed to determine the soundness of the latter view have led to varying conclusions. Meyer¹ and Schloss² observed an increase in the outflow of the coronary vein in the intact heart following the administration of nitrites. Bond,³ on the other hand, failed to get positive results. Voegtlin and Macht⁴ demonstrated a relaxation of coronary arterial rings from the ox and pig that were suspended in oxygenated Locke's solution containing amyl nitrite, sodium nitrite, and erythrol tetranitrite. Loeb⁵ concluded that amyl nitrite had very little effect on the coronary vessels of the profused heart.

In the work here reported, observations were made on the action of sodium nitrite and nitroglycerin on the coronary arteries of the dog.

Method—Healthy young dogs weighing from 12 to 16 kilograms were used in this work. They were anesthetized with ether. An attempt was made to eliminate as far as possible factors which might produce a fall in the arterial pressure, such as profound anesthesia, excessive traumatization of the tissues in the surgical field and hemorrhage. The heart was exposed by sectioning the second, third, fourth and fifth ribs at the left costosternal margin and extending the incision lateralward in the fifth interspace. The free ribs and the sternum were well retracted and a good exposure of the heart was obtained. A slit was made in the anterior wall of the pericardium and extended from the apex upward to the reflection over the great vessels. The heart was

* From the Department of Internal Medicine Rush Medical College and the Presbyterian Hospital.

1 Meyer. Quoted from Sollmann, Manual of Pharmacology, Philadelphia and London, W. B. Saunders Company, 1917.

2 Schloss, Karl. Deutsch Arch f klin Med **111** 310, 1913.

3 Bond, G. S. J Exper M **12** 575, 1910.

4 Voegtlin, C., and Macht, D. I. J Pharmacol & Exper Therap **5** 77, 1913.

5 Loeb, O. Arch f Exper Path u Pharmacol **51** 64, 1903.

slightly suspended by stitching the slit edges of the pericardium to the sternum and the retracted ribs. The left carotid artery was connected with a mercury manometer.

Observations were made on the action of nitroglycerin on the collateral circulation of the distal branches of the left coronary artery. The presence of collateral circulation between the distal branches of the left coronary artery was in a measure determined by noting the extent and degree of cyanosis that appeared distal to the point of ligation of one of these branches. In those instances in which the cyanosis was extensive and of high grade the probabilities of collateral circulation were considered to be slight. In those instances in which the cyanosis was extensive and of high grade the probabilities of collateral circulation were considered to be slight. In those, however, in which the area of cyanosis seemed small we felt justified in assuming that there were probably communicating branches with adjacent vessels. It occurred to us that any dilating action of nitrites on coronary arteries with well established collateral circulation would be manifested by fading of the cyanosis distal to the point of ligation in this vessel. A distal branch of the left coronary artery was thus ligated. As soon as the infarcted area became well demarcated, nitroglycerin $\frac{1}{200}$ grain, was injected into the left ventricular cavity. Careful observations were then made of any change in the color of the infarcted area by two or more independent observers. Nitroglycerin was employed because of the relatively short duration of its action. It was thus considered that any possible change in the cyanotic area would, perhaps, be more apparent.

In other experiments the rate of outflow from the distal branches of the left coronary artery was determined following the administration of sodium nitrite. The artery chosen for the observation was ligated with its accompanying vein at a point as far peripheral as possible. The artery was then carefully dissected free from the cardiac musculature from the point of ligation proximally for a distance of from 1.5 to 2 cm. This was done with as little manipulation and traumatization as possible. The artery was then divided just proximal to the ligation. The wound in the cardiac musculature produced by the dissection was closed by fine silk sutures. The repair of the wound in this manner held the dissected artery up in a position such that the flow from it could readily be caught in a graduate. The flow was collected for thirty seconds prior to and following the administration of 1 gm sodium nitrite. In each instance three or four determinations were made and the average of these taken. Clots often formed at the sectioned end of the artery and interfered with the obtaining of uniform readings. This difficulty was overcome as far as possible by gently milking the vessel prior to each reading.

In some instances the arterial end was freshened by small sharp scissors. At no time was more than 1 mm cut away, and it is not believed that this procedure modified the flow to any significant extent.

Observations on Collateral Circulation—In fifteen dogs observations were made on the effect of nitroglycerin on the collateral circulation of the distal branches of the left coronary artery. In five there was a distinct change in the area of cyanosis distal to the point in which the artery chosen for study was ligated. In four of these the cyanosis practically disappeared within one minute following the administration of the nitroglycerin and coincident with the drop in arterial pressure. The cyanosis returned to its original intensity in from five to seven minutes with the termination of the nitroglycerin action, as indicated by the rise of blood pressure. In each of these five instances the area of cyanosis produced by the ligation was unusually small and the line of demarcation was not very distinct. In six cases questionable results were obtained, and in the remaining four there was no apparent change in the degree of cyanosis following the administration of nitroglycerin. In these ten dogs the cyanotic area seemed relatively large. This was especially true in the latter four.

OBSERVATIONS ON CORONARY OUTFLOW (EXPERIMENT 6)

Reading	Blood Flow in Cc for Period of 30 Sec	Blood Pressure	Time	Remarks
First	12 25	120	10 00 a m	Vessel milked
Second	12 00	120	10 05 a m	
Third	12 40	120	10 08 a m	
1 grain sodium ni- trite administered				
First	14 50	80	10 13 32	Vessel milked
Second	13 25	80	10 17 a m	End of vessel clipped End of vessel clipped
Third	14 1	80	10 28 a m	
Fourth	12 1	115	10 62 a m	
Fifth	11 6	114	11 05 a m	

Observation on Coronary Outflow—In fourteen other dogs the flow from corresponding distal branches of the left coronary artery was determined before and after the administration of sodium nitrite. In six there was an increase in the rate of flow within two minutes following the injection of the nitrite and this with a drop in the arterial pressure of from thirty to forty points. The accompanying table shows the results of one experiment. In three of these the blood pressure returned to approximately the initial reading in forty-five, fifty and sixty-five minutes respectively. The rate of flow was again determined and the results corresponded fairly well with that made prior to the administration of the nitrite. In the other three, in which there was an increased flow the arterial pressure failed to

return to the initial level after periods of more than an hour. In three the rate of flow remained about the same and in five there was a definite decrease in the output of the coronary vessel following the fall in arterial pressure subsequent to the injection of the sodium nitrite. The negative results in four of the latter were possibly due to faulty technique. In one there was considerable delay in adjusting the mercury manometer. The animal had been under anesthesia for more than an hour before the sodium nitrite was given and the arterial pressure had already begun to fall. In three difficulty was encountered in dissecting the vessel. Here again the period of anesthesia was prolonged. It was also noted that excessive manipulation of the heart in the preparation of the vessel caused marked fluctuations in the blood pressure.

COMMENT

It was noted that there was a wide variation in the extent and degree of the cyanosis that appeared distal to the point of the ligation of corresponding distal branches of the left coronary artery. In some former work⁶ in which dogs were allowed to live following the closure of various branches of the left coronary artery there were likewise striking variations in the extent of the cardiac damage found at necropsy. These results indicate that the extent of the collateral circulation between corresponding branches of the left coronary artery varies markedly in the dog. In some instances it would seem that the collateral circulation is well established, while in others very little if any exists. In those in which the area of cyanosis was relatively small the blue color faded following the administration of nitroglycerin. In those, however, in which the area of cyanosis was large there was very little if any change after the injection of nitroglycerin. The former observations seem to indicate that there were communicating branches with the adjacent vessels which were dilated by the nitroglycerin. In the latter instance it was concluded that the collateral circulation was very limited and thus no striking change was anticipated from the nitroglycerin.

In five experiments there was a definite increase in the rate of flow from distal branches of the left coronary artery after the injection of sodium nitrite into the left ventricular cavity, coincident with a drop in the arterial pressure of from 35 to 60 mm Hg in the arterial pressure. According to Wiggers⁷ the coronary circulation is

6 Smith, F. M. The Ligation of Coronary Arteries with Electrocardiographic Study, *Arch. Int. Med.* **22**: 8 (July) 1918.

7 Wiggers, C. J. *Circulation in Health and Disease*, Lea & Febiger, New York and Philadelphia, 1915, p. 84.

dependent to a marked extent on the arterial pressure. The results in these five instances thus indicate that the coronary arteries were dilated by the sodium nitrite. It is difficult to explain the results in the three instances in which the rate of flow remained the same after the blood pressure had fallen except by assuming that the coronary artery was dilated. The conclusions derived from these results are thus in accordance with those held by Meyer, Schloss and Voegtlin and Macht, namely that the nitrites dilate the coronary arteries.

SUMMARY

The action of nitroglycerin on the collateral circulation between distal branches of the left coronary artery was studied in fifteen dogs. In five instances the area of cyanosis that appeared distal to the point of closure of one of these vessels definitely faded following the administration of nitroglycerin. In six the results were questionable and in four they were apparently negative. The observations in the former five indicated that there was a communication with the adjacent vessels which was dilated by the nitroglycerin. In the latter ten it was concluded that very little collateral circulation existed.

In fourteen dogs the rate of blood flow from distal branches of the left coronary artery was determined before and after the administration of sodium nitrite. In six there was a definite increase in the outflow. In three the rate remained about the same and in four it was decreased.

A SIMPLE AND ACCURATE METABOLISM SPIROMETER

SPIROMETER MEASUREMENT OF OXYGEN CONSUMPTION BY THE REBREATHING METHOD *

CHARLES CLAUDE GUTHRIE, M D, P H D

PITTSBURGH

As is commonly known, the essentials of rebreathing types of spirometers comprise a distensible closed space, as an elastic bag or spirometer, a supply of oxygen and means for introducing it into the apparatus, an absorber for removing carbon dioxide, connection for a mouth or nose piece or mask, and means for measuring volume changes in the gas. The subject rebreathes for a definite period, usually ten minutes, and the volume change of gas in the apparatus, after correction for pressure, temperature, and water vapor, indicates the amount of oxygen consumed.

Spirometer—The spirometer shown in Figure 1 is very satisfactory. It consists of a vital capacity type of spirometer (floating bell form, Fig 1, A), a soda-lime carbon dioxide absorber (Fig 1, B), a four way tap (Fig 1, C), a gas mixing pump (Fig 1, D), and a tank of oxygen.

The three point suspension of the bell is very desirable. The bell moves vertically with little side sway or rotation and the scale, which is attached to the bell, remains directly behind the reading sight. The sight (Fig 1, E) is adjustable and may be set instantly against the zero mark of the scale. The scale on one side is numbered from below upward, so that the final reading directly represents the oxygen consumed, no subtraction or other calculation being required. The smallest scale divisions represent 20 c c.

An important feature is the cap on top of the bell and the extension of the breathing pipe almost to the under surface of the cap when the bell is in the lowest position. The cap and the extension tube prevent accidental entrance of water into the breathing pipe. To further guard against water from entering the absorber, the outer end of the breathing pipe is directed upward for some inches. A small brass tap is attached to the lowest point of the pipe and serves the triple purpose of providing a drain, of introducing oxygen, or of withdrawing samples of gas for analysis.

* From the Department of Physiology and Pharmacology, School of Medicine, University of Pittsburgh.

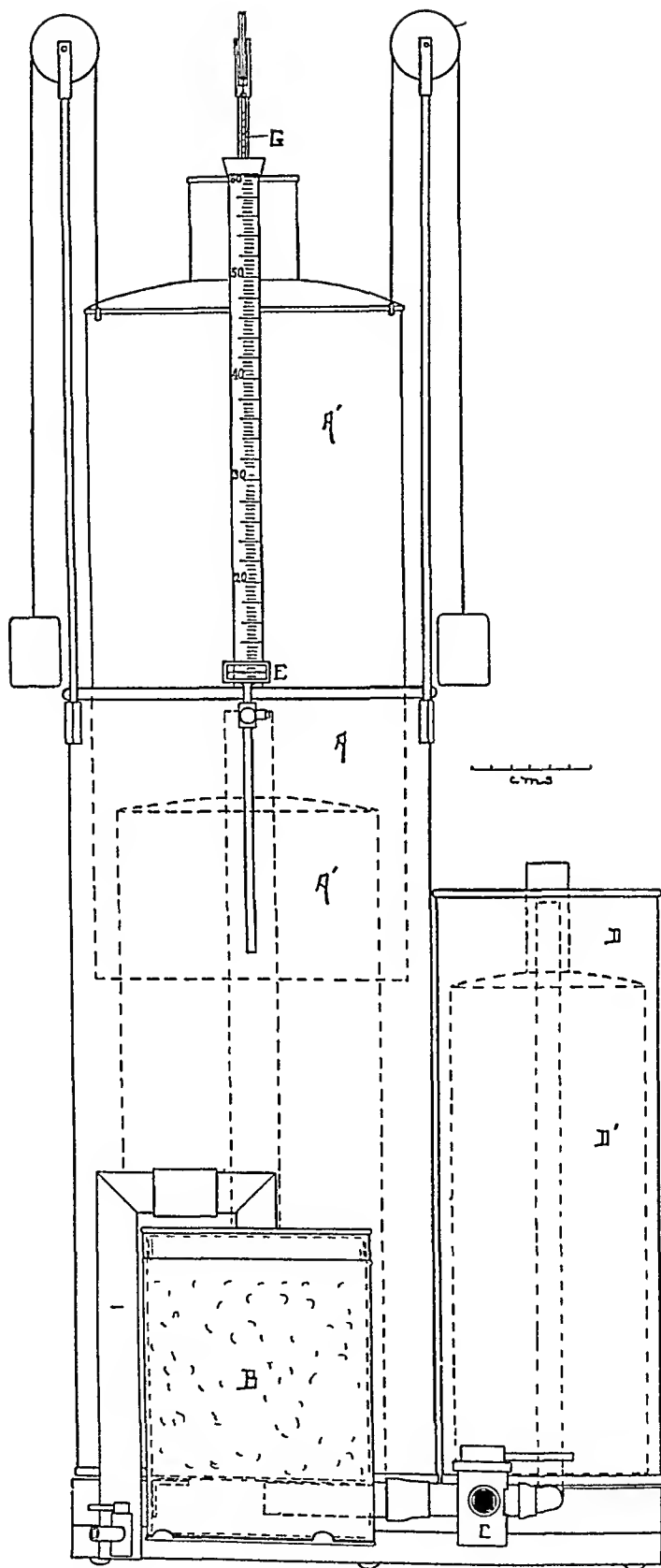


Fig 1 — Closed circuit spirometer for measuring oxygen consumption
A, jacket, A' and A', bell and inside can of spirometer jacket, respectively,
B, carbon dioxide absorber, C, four-way tap, D, mixing pump, D', bell of pump,
E, adjustable sight, G, thermometer

The design of the sight for reading the scale, viz a fine cross wire set in a flattened tube, permits rapid and very accurate readings

Absorber—The form of absorber shown in Figure 1, B gives very satisfactory results. It consists of a copper can provided with a removable top. A tube is attached in the center of the top and another passes horizontally through the side of the can near the bottom to a point

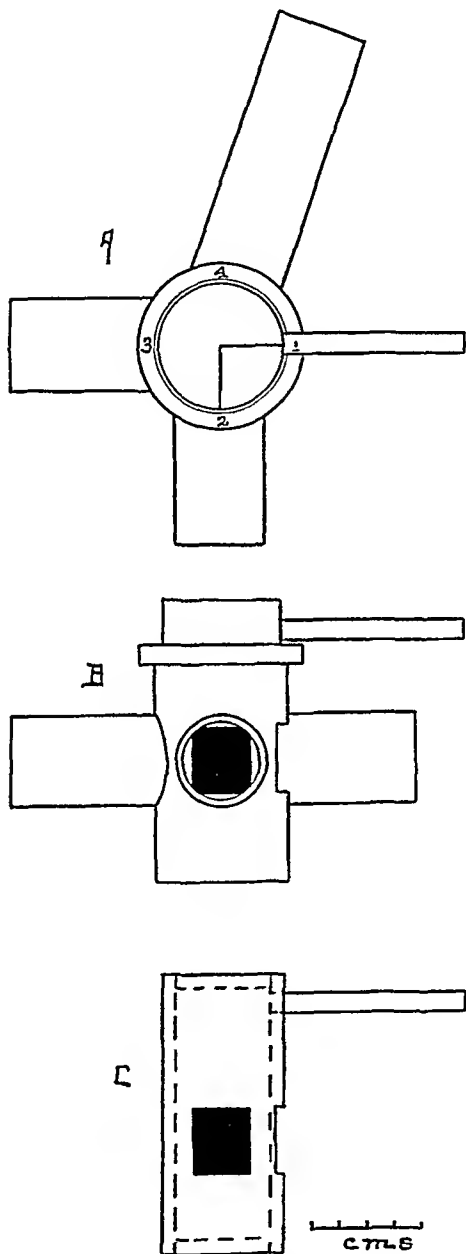


Fig 2—Four way tap A, tap, B, front, C, plug

about half an inch above the center of the bottom. These tubes are for connecting the absorber with the spirometer and with the subject. A false bottom consisting of a close fitting brass wire cloth disc is supported about an inch from the bottom so that the lower tube opens into an air space and is protected from contact with the soda-lime, with

which the can is filled from the false bottom to within an inch of the top. Thus air pressures above and below the ends of the cylindrical mass of absorbing material are uniform. The lid is sealed by means of a strip of adhesive tape or by painting with melted paraffin. The top is connected to the spirometer pipe by a short coupling of tight fitting rubber tubing. The lower tube is connected with the four way tap.

Tap—The tap (Fig 2) serves to connect the spirometer through the absorber with the pump, the subject, or the outside air. The rotating portion of the tap is hollow and provided with two openings spaced 90 degrees apart.

In the first position of the tap, the absorber is shut off and the subject breathes room air. Position two is a quarter turn (clockwise, i. e. to the right) and switches the subject to the absorber and thus to the air in the spirometer, at the same time closing the opening to the outside. Position three connects the absorber with the pump, and position four connects the pump with the outside air.

Pump—The pump essentially is a miniature spirometer of the form described, minus counterpoise weights, etc (Fig 1, D). It is mounted alongside the spirometer and connected with the absorber by means of the four way tap. At the end of the observation the bell of the pump is lifted and lowered by hand until the gas volume becomes stabilized. Before the initial or zero reading and again before the final reading the tap is turned to position three, which connects the spirometer and pump and the air is passed back and forth through the absorber. This soon equalizes and stabilizes temperature and water vapor conditions and ensures removal of all carbon dioxide.

Method of Using—The tap is turned to position four to ensure that the pressure in the pump connections is atmospheric. It is then turned (clockwise) to position two (spirometer with air through subject connector) and the spirometer bell set to read say 1,000 c c, the sight having been set as low as it will go. The tap is turned (clockwise) to position three. The tube from the oxygen tank is connected to the filling tap and the tap opened slowly to let in oxygen until zero on the scale is about 100 c c above the sight. The oxygen tank and the filling taps are closed. The sight is set on the zero mark and the pump bell raised and lowered five times. The scale is read. The air is pumped again and the scale read and this is continued until the readings check. The tap is turned (clockwise) to position four and then to position one. The sight is set on zero, the temperature noted and the subject connected. When breathing is regular, at the end of an expiration the tap is turned (clockwise) to position two and the time noted. A wisp of cotton held near the opening in the tap serves as an index of the respiratory stages. To terminate the test, the spirometer bell is

observed and during a period of regular breathing, at the instant the bell reaches the top of an excursion (end of expiration) the tap is turned (clockwise) to position three, the time noted and the subject disconnected. The scale is read and the air pumped and read and this is continued until the readings check. The temperature is noted.

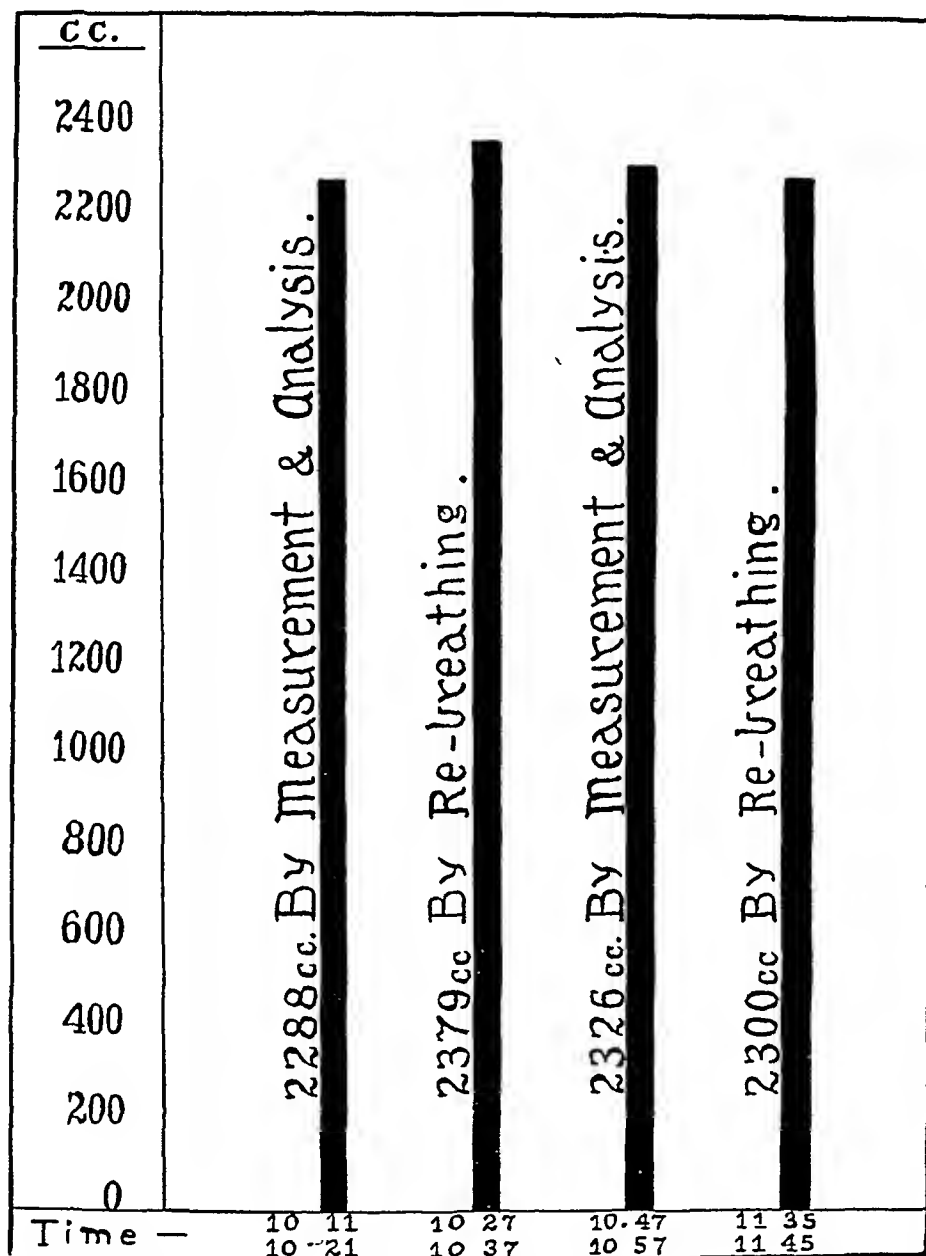


Fig 3—Oxygen consumption as measured alternately by a Tissot form and the closed circuit form of spirometer. Water valves were used with the Tissot form.

The design of the sight is well adapted to catch readings near the beginning and near the end of the test if desired. Such readings are desirable as checks, and in no way interfere with the stationary readings described.

For a new test, the sight is set at the lowest point. The tap is turned to position two. The mouthpiece to the subject being disconnected, by raising and lowering the spirometer bell by hand, the air remaining from the previous test is expelled and the spirometer refilled with fresh air and oxygen as described above.

COMMENT

Under stabilized conditions, as uniform room temperature and absence of drafts, and with good soda lime, changes in the initial total volume readings induced by circulating air through the absorber range from zero to 20 c c, while the changes induced in the final reading usually range from about 20 to 40 c c. Under such conditions results accurate to within less than two per cent can be obtained with an ordinary vital capacity type of spirometer, a soda lime absorber and a two way tap. An ordinary hot water bag can be connected on the tube to the subject and used in place of the pump. By raising and lowering the spirometer bell by hand the air is circulated through the absorber and thus error can be largely eliminated.

Different samples of soda-lime purchased in the open market show great variations in carbon dioxide absorbing powers, some samples being several times more efficient than others. The most satisfactory method is analysis of the gas from the spirometer at the end of the test. With a satisfactory sample of soda-lime not more than a trace of carbon dioxide should be present.

The results from a series of alternate observations made by measuring and analysing the expired air by means of a large bell spirometer and gas analyser and the results obtained with the rebreathing method show the range of the normal variations in successive tests as well as the similarity of the results by the two methods (Fig 3). Sometimes successive observations by either method may give results varying not more than 10 c c for a ten minute period but uniformity within such narrow limits is not to be expected physiologically. Nor is it reasonable to assume corresponding uniformity of air volume in the lungs at the beginning and end of the test.

It may be of interest to note, in conclusion, that the sheet metal work on the spirometer, absorber and pump was done by an ordinary tinsmith and the total cost at regular rates for material (copper) and labor was \$21. The other parts were made in our shop.

SUMMARY

A convenient and accurate form of closed circuit spirometer is described.

THE EFFECT OF THE INGESTION OF FOOD-STUFFS ON THE RESPIRATORY EXCHANGE IN PULMONARY TUBERCULOSIS¹

WILLIAM S McCANN, M D

With the technical assistance of G F Soderstrom

NEW YORK

Elaborate studies of the effect of foodstuffs on the respiratory metabolism have been made by Rubner¹ and Lusk,² who are the principal workers in this field. Reference should be made to the latter for an excellent review of the subject. These investigations were concerned chiefly with the calorimetric observations, in which the magnitude of pulmonary ventilation was not measured.

In a previous communication³ the effect of protein ingestion on the heat production of two tuberculous patients and two normal subjects was found to be identical. On a priori grounds the assumption was made that the pulmonary ventilation varies directly with the metabolism. Confirmatory evidence on this point is given by Peabody⁴ who shows the parallelism between the minute volume of air expired and the level of metabolism in patients with exophthalmic goiter. The general validity of the assumption is well demonstrated by Pearce,⁵ who showed that total ventilation and cardiac volume output, within the capacity of the organism, vary directly with the rate of oxygen consumption.

Since the problem of selecting a proper diet for patients with pulmonary tuberculosis involves a knowledge of the effect of food ingestion on the function of the diseased lungs, it seems important to present the data of a few experiments bearing on this point.

Technic —The apparatus used for this work was a Tissot spirometer. This spirometer has a capacity of eighty-four liters. The bell is counterpoised by a container into which water siphons, so that as the buoyancy of the bell diminishes the weight of the counterpoise increases by an equivalent amount. A description of this device is to be found in the monograph by Carpenter.⁶

* From the Russell Sage Institute of Pathology in affiliation with the Second Medical Division of Bellevue Hospital.

1 Rubner, M. *Die Gesetze des Energie-verbrauchs bei der Ernährung*, 1902.

2 Lusk, G. *Science of Nutrition*, Ed 3, 1917, pp 223-247.

3 McCann, W S, and Barr, D P. *Clinical Calorimetry*, Paper No 29, *The Metabolism in Tuberculosis*, Arch Int Med 26 663 (Nov) 1920.

4 Peabody, F. *Respiration in Disease*, Oxford Medicine, 1 409.

5 Pearce, R G. *The Cardiorespiratory Mechanism in Health and Disease*, Arch Int Med 27 139 (Jan) 1921.

6 Carpenter, T M. *A Comparison of the Methods for the Study of the Respiratory Exchange*, Carnegie Inst of Washington, D C Pub No 216.

The wire connecting the spirometer bell and the counterpoise runs over a pulley 12 inches in diameter. On this pulley are graduation marks for each liter. Readings are made to the first place of decimals. These calibrations were made with a Bohr meter at 23 C. Since calculations were to be based on dry gas volume, it was necessary to apply calibration corrections. These corrections were made as follows. A metal bomb containing dry oxygen was weighed. The gas was then allowed to escape, passing through water into the spirometer. At intervals of five liters on the spirometer scale the bomb was closed and again weighed. The difference in weight equals the weight of oxygen admitted to the spirometer. The temperature of the spirometer and the barometer reading were noted. The observed reading of the gas volume in the spirometer was then corrected for aqueous vapor tension at the observed temperature, to 0 C and 760 mm dry. This dry gas volume was then compared with the volume which the known weight of dry oxygen gas would occupy at 0 C and 760 mm dry. This is obtained by multiplying the weight of oxygen by 22.4/32. In this way a calibration curve was constructed. The oxygen used contained about 0.4 per cent chiefly argon.

The valve used to separate inspired and expired air was made by G. F. Soderstrom. A sketch of this valve is shown in the accompanying figure. After having devised the valve it was found that a similar valve had been described by Tigerstedt.⁷ The mouthpiece used was identical with that of the American gas masks.⁸ The nose was then closed with a spring clip.

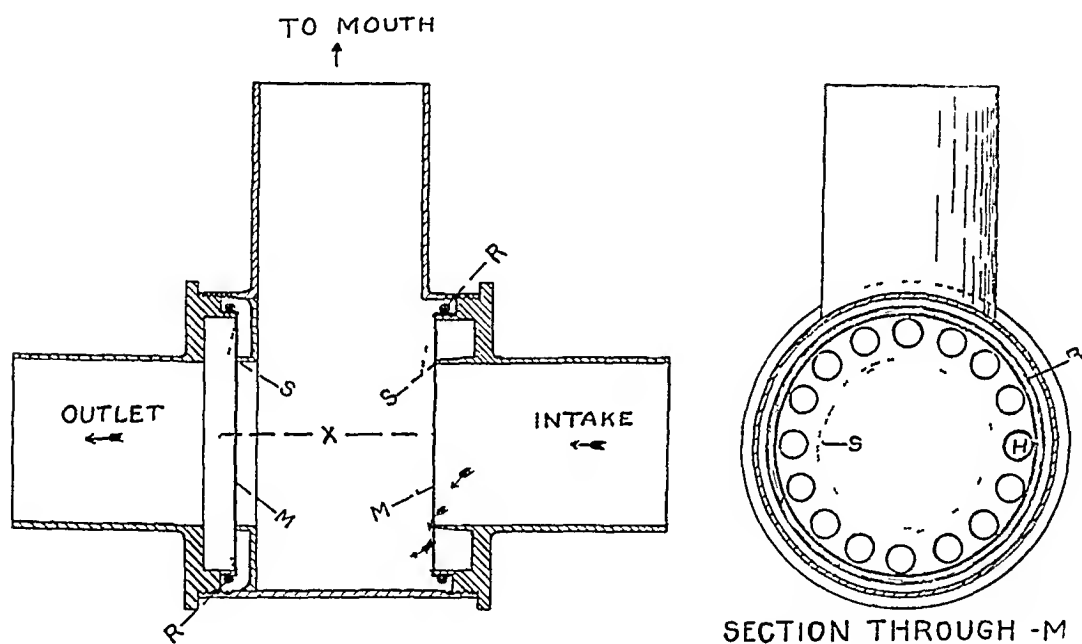
Patients were prepared in the usual manner for basal metabolism determinations. No food was allowed after 4.30 or 5 p. m. on the preceding day so that the basal determinations were generally made at least sixteen hours after eating. A period of absolute rest of twenty minutes or more before the test was observed in all bed patients and thirty minutes at least of absolute rest reclining in a steamer chair for all normal subjects or ambulatory patients.

Many persons on the first occasion of breathing through the mouth, with the nose closed by a clip, will alter their breathing from the normal type. The invariable tendency is to over ventilate either by increasing the depth of breathing, or by increasing the rate. Some patients can never be trained not to do this. In the experiments given here subjects were allowed to become accustomed to the apparatus before the beginning of the experiment. The rate of respiration was counted before

⁷ Tigerstedt, R. *Lehrbuch der Physiologie des Menschen*, Leipzig, 189, 1902. The valve is ascribed to Loven, but the original reference is not given.

⁸ This was obtained from the Siola Rubber Manufacturing Co., 103 Park Ave., New York.

inserting the mouthpiece and the normal type of breathing was closely observed. After adjusting the mouthpiece and nose clip the respirations were again observed until they again became of normal rate and type. At this time 8 or 10 liters of expired air were admitted into the spirometer in order to get the approximate minute volume and to fill the dead space of the apparatus with air of the same composition as that to be measured and analyzed. This gas was expelled as completely as possible, i.e. to zero mark. If the subject continued to breathe normally, the three-way valve opening into the spirometer was thrown open at the end of an expiration and the time noted. After from six to ten minutes, depending on the minute volume expired, the valve was closed at the end of expiration and the time noted.



Modified Loven valve. *M* and *M'* are very thin rubber membrane which in the closed position is in contact with the rims *S* of the valve seats. The membranes are held in place by the rings *R*, which slip on and off. The holes in the membrane *H*, are burned through the rubber after it is fastened in place. Contact of *M* with *S* is made more secure by moistening the membrane with glycerin. *X* shows the open position of membranes, which open and close reciprocally.

Movement on the part of the patient, distress from tickling sensations in throat or a desire to cough were made matters of note. The manner of closure of the lips over the mouthpiece was carefully watched. If there was any doubt as to the possibility of leaks about the lips or nostrils a lather of shaving soap was applied. A leak would be detected by the formation of a bubble.

It will be seen, therefore, that the valve which was used required considerable care. For this study it was preferred to the other forms in use because of its small dead space, the ease with which it opened

TABLE 1—DATA ON FIVE NORMAL SUBJECTS AND FIVE TUBERCULOSIS PATIENTS

Name, Age, Sex, Surf Area, Ht -Wt Formula	Date	Volume Expired, Liters S.T.P.D. per Hr	Gas Analysis		Calories per Hr	R Q	Metab olism Variation from Av N ± %	Temp, Degree F	Pulse Rate per Min	Resp Rate per Min	Urine N per Hr, Gm	Remarks	Diagnosis
John S 21 yrs 1 69 Sq M	9/ 3/20	274 6	{4 08 {4 05	O ₂ % 10 51} 16 55}	60 4	0 898	-- 9		68	16		Basal Ingested 350 gm lean beef + 10 gm butter 127 min after meal	Normal control
	9/ 3/20	384 8	3 59	16 51	84 6	0 766	+27 5		62	16		116 min after meal	
	9/10/20	332 1	{4 17 {4 12	16 16} 16 17}	79 0	0 836	+19					296 min after meal	
	9/10/20	292 1	4 17	15 86	73 6	0 780	+11		64	12		Basal	Acute pneumonic phthisis; extensive bilateral involve ment
	11/29/20	578 5	{2 20 {2 17	18 40} 18 42}	72 7	0 824	+30	101 6	108	36		Basal Ingested 140 gm fat in cream	
Abr M 21 yrs 1 42 Sq M	11/30/20	554 4	{2 11} {2 14}					101 4	108	36		100 min after meal	
	11/30/20	613 0	{2 24 {2 25	18 30} 18 29}	80 3	0 807	+43	100 6	120	34		139 min after	
	12/ 3/20	662 8	{2 07} {2 09}	18 44	82 1	0 782	+47	100 4	135	44		296 min after	
	12/ 3/20	656 2	{1 92} {1 95}	18 42	81 8	0 715	+46	102 8	129	40		Basal	Pulmonary tubercu losis, very extensive bilateral infltra- tion with numerous cavities throughout
	12/ 2/20	540 5	{2 14 {2 17	18 50} 18 50}	65 5	0 848	+12	98 0	80	30		Basal Ingested 290 gm lean beef + 6 gm butter 76 min after meal	
Fred R 41 yrs 1 62 Sq M	12/11/20	532 2	{2 27 {2 21	18 20} 18 21}	75 0	0 770	+28	100 0	102	30		206 min after	
	12/11/20	608 7	{2 51} {2 51}					100 8	114	30		Basal Slight over- ventilation, ingested 150 gm lean beef 103 min after meal	Normal
	12/11/20	664 4	2 49	18 17	91 6	0 840	+57	100 8	114	34			
	12/30/20	266 2	{4 18 {4 21	16 46} 16 41}	59 7	0 913	+ 3		66	12	0 483		
	12/30/20	277 6	{4 08 {4 08	16 57} 16 53}	60 8	0 906	+ 5		68	12	0 052		
C H L 26 yrs 1 56 Sq M	12/30/20	269 0	4 09	16 16	60 1	0 886					0 567	256 min after	
	12/30/20	245 6	4 26	15 89	61 6	0 804	+ 7				0 465	335 min after	

W 26 yrs 1 91 Sq M	12/31/20	304.2	{4.26 {4.27	15.97 15.99	75.0	0.825	0				60	12	2.145	Basal Ingested 460 gm lean beef	Normal
	12/31/20	341.2	4.23	16.17	81.0	0.855	+7				60	12	0.247	80 min after meal	
	12/31/20	337.1	4.25	15.93	84.0	0.810	+11				60	12	0.247	149 min after	
	12/31/20	349.6	4.03	16.13	83.5	0.798	+11				60	12	0.247	210 min after	
J O'C 49 yrs 1 50 Sq M	12/14/20	434.0	{2.42 {2.42	18.04 18.07	62.0	0.806	+3	100.8			72	24		Basal	Tuberculosis, pulmonary, moderately advanced infiltration of both upper lobes
	12/23/20	402.0	{2.49 {2.49	18.42 18.40	61.0	0.969	0	100.6			72	27		Basal Constant desire to cough, ingested 100 gm lean beef + 3 gm butter 74 min after, desires to cough	
	12/23/20	389.0	{2.82 {2.82	17.96 17.93	58.0	0.921	-4	100.8			75	24		152 min after, breathing normally	
	12/23/20	411.0	{2.46 {2.44	18.23 18.22	55.3	0.874	-8	101.4			72	24		Basal Ingested 350 gm lean beef	Pulmonary tuberculosis, advanced bilateral infiltration with large cavities, fistula in ano
Geo R 35 yrs 1 62 Sq M	1/26/21	434.2	{2.73 {2.74	17.76 17.72	67.6	0.816	+6	98.8			75	16		77 min after meal	
	2/ 8/21	523.0	{2.87 {2.83	17.64 17.63	8.60	0.822	+33	99.4			88	20		188 min after	
	2/ 8/21	544.2	{2.73 {2.73	17.73 17.71	87.0	0.803	+34	99.6			80	25			
J V B 13 yrs 1 51 Sq M	5/13/21	187.7	{2.56 {2.58	17.85 17.85	74.9	0.789	+29	99.8			80	19		Fasting, not basal Slight movement, body tense ingested 102 gm glucose in "Kaffee Hag" 1 hours after meal	Pulmonary tuberculosis, dense infiltration of right upper and middle lobes with multiple excavations, large cavity left upper lobe, below which consolidation with small cavities
	5/18/21	573.4	{2.56 {2.53	18.37 18.36	73.9	0.969		100.4			108	21		90 min after	Control
	5/18/21	553.5	2.63	18.25	74.3	0.963		100.4			108	18			
G M 32 yrs 1 57 Sq M	5/29/21	203.8	{3.91 {3.93	15.82 15.86	51.3	0.723	-11	97.8			70	7		Basal Ingested 100 gm glucose in "Kaffee Hag" 37 min after, changed type of breathing 87 min after, quiet normal resp	
	5/29/21	212.0	3.81	16.15	57.5	0.750	0				76	12			
	5/29/21	269.4	{3.88 {3.84	16.00 16.64	57.7	0.863	0				76	9			
Wm U 12 yrs 1 65 Sq M	1/10/21	227.6	{3.22 {3.22	16.81 16.85	46.5	0.735	-27	94.0			42	9	0.346	Basal End of 16 days, fasting, wt 51.7 kg, 1.65 sq m, ingested 105 gm protein 53 min after meal	Normal man physiologically, control, voluntary subject of fasting experiment
	1/10/21	273.6	{3.73 {3.70	16.28 16.32	62.9	0.754	-1					9	0.142		
	1/10/21	304.6	{3.56 {3.52	16.57 16.60	65.7	0.769	+3.5				64	8	0.580	110 min after	
	1/10/21	501.4	{3.51 {3.52	16.12 16.43	67.0	0.734	+5.4	99.0			66	9	0.490	209 min after	

(pressure of from 1 to 2 mm water) and the complete lack of leakage back. The absence of these virtues might have made considerable difference in the volume of air respired.

Samples of air from the spirometer were drawn into special sampling bottles⁹ over a solution consisting of equal parts of glycerin and saturated sodium chlorid solution. The sample of air can be kept unchanged for at least twenty-four hours over this solution¹⁰. In our hands this has been fully as satisfactory as mercury and much cheaper.

The air analyses were made with a modification of Henderson's¹¹ air analyser. In its original form the apparatus had a very short water jacket. In our apparatus a long water jacket was used and in place of the original absorber shell for carbon dioxid absorption a bulb was used very much as in the Haldane apparatus. These are minor details. The essential feature of the apparatus is the very ingenious four-way stopcock of Henderson.

Gas burets were recalibrated with mercury and calibrations and technic were checked by analyses of pure outside air. The values obtained for carbon dioxid vary from 0.02 to 0.03 per cent and for oxygen from 20.93 to 20.97 per cent. In calculation Haldane's value for oxygen in outside air was used, 20.93 per cent. In analyses of expired air duplicate oxygen analyses were checked within 0.04 per cent except in one or two cases. The greatest variation allowable between duplicates is 0.06 per cent.

A description of the calculation is given very clearly by Boothby and Sandiford¹². We have used a slightly different set of tables for correction of gas volumes. They may be found in convenient form by Wells¹³.

DISCUSSION OF RESULTS

Experimental data are given in Table 1. Ten subjects were used, five of whom were normal. The most striking thing on first inspection of this table is the difference between tuberculous and normal individuals in volume expired per unit of time. The explanation of this is quite obviously to be found in the reduced vital capacity of the tuberculous patients. Peabody¹⁴ has shown that normal individuals may increase

⁹ Devised by Dr. Donald Van Slyke of the Rockefeller Institute for Medical Research.

¹⁰ We are indebted to Dr. Wm. C. Stadie for this point in technic.

¹¹ Henderson, Y. The Haldane Gas Analyser, *J. Biol. Chem.* **33**, 31, 1918.

¹² Boothby, W. M., and Sandiford, I. Basal Metabolic Rate Determinations, 1920, W. B. Saunders Company, Philadelphia.

¹³ Wells, H. L. Tables for Chemical Calculations, 1903, Henry Holt, New York, p. 10.

¹⁴ Peabody, F. Cardiac Dyspnea, *A. J. M. Sc.* **155**, 100, 1918.

the tidal air up to one third of the vital capacity, beyond which point further increase in respiratory exchange must be accomplished by increasing the rate of respiration. Since the dead space has a relatively constant volume, increasing the rate of respiration decreases the ratio of effective minute volume to total minute volume expired. This results in a lower percentage of carbon dioxide and a higher percentage of oxygen in expired air, other factors being equal.

Referring to Table 2 it will be seen that for each of the five normals and five patients the minute volume and tidal air have been tabulated from the data for each basal period. For the normals the average minute volume was 4.25 liters, average respirations were 11 per minute and average tidal air 397 c c. This is 11 per cent of the average normal vital capacity as estimated from the surface area by the formula of

TABLE 2—MINUTE VOLUME AND TIDAL AIR

Name	Volume Expired, Basal Liters per Hr	Resp per Min	Minute Volume, C c Expired	Tidal Air, C c	Effective Minute Volume, C c *	CO ₂ C c per Min Pro-duced	Ratio of Total Min Vol to CO ₂ per Min	Ratio of Effective Min Vol to CO ₂ per Min
Normal								
John S	274.6	16	4,577	286	2,497	185	24.7	13.5
O. H. L.	266.2	12	4,437	370	2,877	185	23.9	15.5
W.	304.2	12	5,070	423	3,510	214	23.7	16.4
G. M.	203.8	7	3,397	485	2,487	132	25.7	18.9
Wm. U.	227.6	9	3,793	421	2,623	121	31.3	21.6
Average		11	4,255	397	2,799	167	25.9	17.2
Tuberculous								
Ab. M.	578.5	36	9,641	263	4,961	203	46.3	23.8
Fred R.	540.5	30	9,008	300	5,103	192	46.9	26.6
J. O. C.	434.0	24	7,233	301	4,113	173	41.8	23.8
Geo. R.	434.2	16	7,237	452	5,153	193	37.5	26.7
J. V. B.	487.7	19	8,128	428	5,653	206	39.5	27.5
Average		25	8,249	350	4,799	195	42.5	25.7

* Effective minute volume = total minute volume minus respirations per minute \times 130 c c. The average dead space assumed to be 130 c c.

West¹⁵ In the tuberculous series the average minute volume was 8.25 liters, average respiration rate 25 per minute and average tidal air 350 c c. The value for the tidal air must be approximately maximal inasmuch as in all of the tuberculous cases increases in metabolism were accompanied by accelerated respirations. If the same ratio of maximal tidal air to vital capacity obtains in tuberculosis as in normals this would indicate an average vital capacity of from 1,000 to 1,100 c c. This figure is undoubtedly lower than these subjects would have shown if the vital capacity were determined in the erect posture. It probably represents the reduced vital capacity of the recumbent posture.

¹⁵ West, H. F. A Comparison of Various Normal Standards for the Normal Vital Capacity of the Lungs, Arch Int Med 25:306 (March) 1920.

In Table 2 the effective minute volume, or alveolar ventilation for each individual has been calculated on the assumption that the dead space was 130 c c. This gives an average effective alveolar ventilation for the five normals of 2,799 c c, and for the five patients of 4,799 c c. However, it will be seen that the average amount of carbon dioxide eliminated per minute was higher in the tuberculous series, 195 c c per minute for the patients against 167 c c per minute for the normals. The ratio of total ventilation to volume carbon dioxide eliminated is almost twice as large for the tuberculous cases as for the normals. Even when they are compared on the basis of the ratio of effective alveolar ventilation to carbon dioxide elimination the ratio is again less for the normals 17.2 for normals and 25.7 for patients. This is in keeping with the results of experiments on the effect of artificially elevating body temperature. Hill and Flack¹⁶ called attention to the overventilation which occurs in subjects immersed in hot baths. A marked fall in alveolar carbon dioxide tension was noted. Haggard¹⁷ has shown that under these circumstances there is a diminution of the ratio ($\text{H}_2\text{CO}_3/\text{NaHCO}_3$), which he believed to be due to the decreased solubility of carbon dioxide at elevated body temperatures. He found that the alveolar carbon dioxide tension was reduced, and that the carbon dioxide combining power of the blood was not correspondingly changed.

It should be pointed out, however, that, in the basal observations which are tabulated in Table 2, the elevation of temperature was in no case very high, so that there may be other unknown factors which enter into the production of the overventilation of these patients.

In Table 3 are tabulated the comparisons of the percentage increases in heat production and total pulmonary ventilation after the ingestion of varying amounts of foodstuffs. It will be seen at once that the parallelism is very close in both normal and tuberculous subjects.

In considering the effect of protein ingestion it will be seen that there is considerable variation in the amount of increase of heat production. There are several factors which determine the extent to which protein will increase heat production. First, the amount of protein relative to the size of the individual subject, second, the previous nutritive condition of the subject or the level of endogenous protein metabolism at the time of ingestion of protein food. Environmental temperature plays a part. The development of our knowledge of these

¹⁶ Hill, L., and Flack, M. The Influence of Hot Baths on Pulse Frequency, Blood Pressure, Body Temperature, Breathing Volume and Alveolar Tension of Man, *J. Physiol.* **38** 57, Proc., 1909.

¹⁷ Haggard, H. W. The Alteration of the Carbon Dioxide Ratio ($\text{H}_2\text{CO}_3/\text{NaHCO}_3$) in the Blood During Elevation of Body Temperature *J. Biol. Chem.* **44** 131, 1920.

factors is the result of the researches of Rubner¹ and Lusk². The amounts of protein ingested have been expressed in terms of grams per square meter of body surface area. In the cases of C, H, L and J, O'C the amount of protein ingested was not sufficient to increase the heat production, it served merely to replace endogenous protein.

External temperature probably plays no part in these experiments since all subjects had sufficient covering to maintain perfect comfort. This gives an environmental temperature of about 33° C at the skin surface. The effect of previous nutritive condition is very strikingly shown in the case of William U, who ingested protein after sixteen days of complete starvation. Subject John S habitually lives on a very low protein diet. Subject W lives on a high protein diet. His nitrogen excretion per hour before ingesting protein was higher than that observed subsequently. Tuberculous subjects Fred R and Geo R behaved similarly to the four subjects studied by McCann and Barr³ in the respiration calorimeter.

TABLE 3—PERCENTAGE INCREASE IN HEAT PRODUCTION AND PULMONARY VENTILATION AFTER EATING

Name	Amount Foodstuff Ingested, Gm	Calories per Hr - Basal	Maximum Calories per Hr after Food Ingestion	Increase Heat Produced, per Cent	Increase Ventilation, per Cent	Time of Maximum Increase Hrs after Ingestion	Grams Protein per Sq Meter Body Surface
John S	73 protein	60.4	84.6	40	40	2	43
W	96 protein	75.0	84.0	12	11	2½	50
Wm U	105 protein	46.5	67.0	44	32	3½	61
C, H, L	31 protein	59.7	61.6	3		5½	20
Fred R	61 protein	75.0	91.6	22	20	3½	40
J, O'C	21 protein	62.0	55.3	(-11)	(-5)	2½	13
Geo R	73 protein	67.6	87.0	29	25	3	45
Ab M	140 fat	72.7	82.1	12	14.5	2½	
G M	100 glucose	53.1	57.7	12	32	1½	
J, V, B	100 glucose	74.9	74.3	0	17	1-1½	

That protein has a specific dynamic action in tuberculosis brings this disease into sharp contrast with typhoid fever. In the latter disease the endogenous protein metabolism is already high at the time of ingesting protein, which merely replaces endogenous protein¹⁸. McCann and Barr³ have shown that the level of protein metabolism in tuberculosis is not high, so that ingestion of protein increases the total oxidative processes.

Lusk,² quoting Rubner, states that when 100 per cent of the basal requirement of energy is ingested as meat there is an average increase

¹⁸ Coleman, W., and DuBois, E. F. Calorimetric Observations on the Metabolism of Typhoid Patients With and Without Food, Arch Int Med 15:2 (Jan) 887 (June) 1915.

of 36.7 per cent in the twenty-four hour heat production, if ingested as fat, the metabolism is raised 12.7 per cent and if as sugar, 5.8 per cent

Murlin and Lusk¹⁹ and Magnus Levy²⁰ find somewhat lower figures for the specific dynamic action of fat than those of Rubner. For each 100 calories of ingested fat the metabolism is increased. Rubner, 12.7 calories, Murlin and Lusk, 4.1 calories, and Magnus Levy, 2.5 calories.

In the case of Abr. M., ingestion of 1,312 calories as fat increased the heat production 12 per cent in from two to five hours. The ingestion of only 410 calories as glucose in the case of J. V. B. caused no increase in metabolism but increased the ventilation 17 per cent, and in the case of G. M. the metabolism rose 12 per cent and the ventilation rate 32 per cent. Thus, it is seen that the greatest number of calories may be ingested in the form of fat with the least effect on the total ventilation. A marked increase in ventilation occurs during carbohydrate oxidation in spite of a small specific dynamic action. This is due to the relatively greater quantities of carbon dioxide to be eliminated, or, in other words, to the higher respiratory quotient of carbohydrate metabolism. Confirmatory evidence on this point has been found in the work of Benedict and Carpenter²¹. These authors studied the effect of the ingestion of many foods on the heat production and gaseous exchange. Unfortunately, in the experiments on fat and protein no data are given regarding the ventilation. However, some of their carbohydrate experiments were made with the Tissot spirometer. They found (p. 231) that the average maximum effects were produced, in the order of greatest to least, by sucrose, levulose, lactose, and dextrose. On page 217, Table 149, subject H. H. A., after ingestion of 100 gm. sucrose the ventilation increased from 4.29 liters per minute to a maximum of 6.86 liters per minute, an increase of 2.57 liters, or 60 per cent. The carbon dioxide elimination increased 55 per cent, while the oxygen consumption rose only 14 per cent.

ADDENDA

Description of Experimental Subjects—It should be emphasized that all of the tuberculous subjects were in an advanced stage of the disease. With the exception of J. V. B., a detailed history will be found for each in a subsequent communication, "The Protein Requirement in Tuberculosis."

¹⁹ Murlin, J. R., and Lusk, G. *J. Biol. Chem.* **22**, 15, 1915.

²⁰ Magnus, L. A. *Arch. f. d. ges. Physiol.* **55**, 1, 1894.

²¹ Benedict, F. G., and Carpenter, T. M. *Food Ingestion and Energy Transformations*, Carnegie Institution of Washington, D. C., Pub. No. 261, pp. 217, 231.

CASE 1—Ab M, an Arabian male, aged 21 years, height 168 cm, weight 40 kg Acute illness of two months' duration with signs of pneumonic consolidation of the entire left lung Many tubercle bacilli in the sputum Temperature range, from 100 to 104 F

CASE 2—Fred R, aged 41 years, height 167 cm, weight 47 kg History of chronic cough and loss of weight for three years' duration Had been hospitalized for eight months Many tubercle bacilli in the sputum Marked emaciation, dyspnea and cyanosis Physical signs of extensive bilateral infiltration with numerous cavities Confirmation by roentgenogram and necropsy

CASE 3—J O'C, a male, aged 49 years, height 174.3 cm, weight 49.7 kg History of probable active pulmonary tuberculosis of eleven years' duration Chronic cough, slow emaciation, and slight hemoptyses Symptoms became acute and urgent only one month before admission Physical signs of infiltration of both upper lobes of lungs, more advanced on the right side, with small amount of fluid in the right pleura Some enlargement of the heart with retraction of the heart to the right Many tubercle bacilli in the sputum Temperature range from 97.4 to 102.2 F

CASE 4—George R, aged 35 years, height 163.5 cm, weight 57 kg History of fistula in ano for three years Pulmonary symptoms of six months' duration Marked constitutional symptoms of only two months' duration—night sweats and loss of weight Physical and fluoroscopic examination showed marked infiltration of both upper lobes with a large cavity in each Sputum contained many tubercle bacilli Temperature range from 98 to 101 F

CASE 5—J V B, a male, aged 43, height 174 cm, weight 44.4 kg Symptoms all developed within six months—cough, night sweats, and a loss of weight of 42 pounds Fluoroscopic examination showed a large cavity in the left upper lobe, below which there was consolidation with multiple excavations The right middle and upper lobes were densely infiltrated, containing multiple excavations There was a similar condition in the apex of the right lower lobe Sputum contained many tubercle bacilli Temperature range from 98 to 102 F

The normals used as controls are described briefly as follows

CASE 6—John S, laboratory assistant, aged 21 years, height 165 cm, weight 61.5 kg Subject was fat Had no symptoms of serious nature during two years in the laboratory Physical examination and urine examination revealed no abnormalities

CASE 7—C H L, a female medical student aged 26 years, height 161.1 cm, weight 53.9 kg Had been in good health during three years of medical course Physical examination negative Urine normal

CASE 8—W, a male medical student, height 179 cm, weight 73 kg Large athletic individual in perfect health Physical examination negative Urine normal

CASE 9—G M, physician, female, aged 32 years, height 160 cm, weight 56.05 kg In perfect health Physical examination normal Urine normal

CASE 10—Wm U, a lawyer, aged 42 years, height 179 cm, weight 51.7 kg Had an abscess in neck in childhood, gonorrhea in 1902, mastoiditis and chronic otitis media from 1914 to 1916, malaria of the quartan type in 1909, in perfect health during experiment He had undertaken a fasting experiment, which he had done several times before Between Nov 28, 1920, and the date of the respiration experiment of Jan 19, 1921, he had fasted forty-two days in three periods of 10, 16 and 16 days respectively The experiment given in Table 1 is the breaking of the last sixteen day fast The meal taken was one of pot cheese which had been washed thoroughly until it was practically free from

carbohydrates The physical examination was normal, except for emaciation due to fasting He was 71 pounds under the weight at the start of fasting, November 28 Urine normal

CONCLUSIONS

1 The total pulmonary ventilation of five cases of advanced pulmonary tuberculosis was approximately twice that of five normal controls The percentage of carbon dioxide produced and of oxygen absorbed, in terms of expired air, was much reduced as compared with normals

2 The alveolar ventilation in the tuberculous patients was greater than that of the normal subjects, as was the ratio of alveolar ventilation to the volume of carbon dioxide expired

3 The ingestion of protein food increased both heat production and total pulmonary ventilation in a corresponding degree in both tuberculous patients and controls After ingestion of 70 gm protein (from 40 to 45 gm per square meter of body surface) heat production was increased from 22 to 29 per cent and ventilation from 20 to 25 per cent in tuberculous subjects

4 In the form of fat the greatest number of calories may be ingested with the least effect on the pulmonary ventilation Carbohydrates increase the ventilation out of all proportion to their effects upon the general oxidative processes and heat production This is believed to be due to the relatively greater quantities of carbon dioxide eliminated during carbohydrate oxidation, in other words, to the higher respiratory quotient

INDEX TO VOLUME 28

	PAGE
Acidosis and capillary poisons, G B Wallace and E J Pellini	711
Alkaptonuria, case of, with study of its metabolism, R B Gibson and C P Howard	632
Anemia, hemorrhagic influence of inorganic iron on regeneration of blood after, J H Musser, Jr	638
Arrhythmia, terminal cardiac arrhythmias, report of 3 cases, F R Dieuaide and E C Davidson	663
Arthritis, intravenous use of foreign protein in chronic cases of arthritis, with special reference to use of secondary proteose, R G Snyder and M A Ramirez	50
Ashby, W, and Rosenow, E C Focal infection and elective localization in etiology of myositis	274
Aub J C, and Means, J H Basal metabolism and specific dynamic action of protein in liver disease	173
Auriculoventricular bundle, further observations on experimental lesions of branches of auriculoventricular bundle of dog, F M Smith	453
Bailey, P, and Bremer, F Experimental diabetes insipidus	773
Baldwin, L B, and Mackenzie, G M Local desensitization in hypersensitive individuals and its bearing on prevention of hay-fever	722
Barach, A L and Woodwell, M N Studies in oxygen therapy, with determinations of blood gases I In cardiac insufficiency and related conditions	367
Studies in oxygen therapy II In pneumonia and its complications	394
Studies in oxygen therapy III In an extreme type of shallow breathing occurring in lethalargic encephalitis	421
Barsky, J, and Kahn, M Acute yellow atrophy of liver	142
Bauman, L Chemistry and clinical significance of urobilin	475
Beeler, C, and Fitz, R Glycemia, glycosuria and water excretion in obesity	804
Betelnut chewing and its effects, including cancer of mouth, A G Ellis	252
Blood causes of variations in sedimentation of corpuscles and formation of crusta philogistica ("size," "buffy coat") on blood, H C Gram	312
comparative studies in chemistry of blood and cerebrospinal fluid, G Egerer-Seham and C E Nixon	561
gases, studies in oxygen therapy, with determinations of blood gases, in cardiac insufficiency and related conditions, A L Barach and M N Woodwell	367
regeneration, influence of inorganic iron in, after hemorrhagic anemia, J H Musser, Jr	638
study of significant chemical changes in blood coincident with malignant tumors, J A Killian and L Kast	813
total nonprotein nitrogen constituents of blood in chronic nephritis with hypertension, J L Williams	426
Bloom, W, and Macht, D I Experimental inquiry into cerebral and neuromuscular manifestations of digitalis	678
Boas, E P and Mann, H Right ventricle in pulmonary tuberculosis	62
Book Reviews	
Diagnostik der Kinderkrankheiten, mit besonderer Berücksichtigung des Säuglings, E Feer	236
Tuberculosis of Children, Its Diagnosis and Treatment, H Much	124
Bremer, F, and Bailey, P Experimental diabetes insipidus	773
Buchanan, J A Study of the S-T interval in 1,028 electrocardiograms	484
Cancer, betelnut chewing and its effects, including cancer of mouth, A G Ellis	252
Capillary poisons and acidosis, G B Wallace and E J Pellini	711
Carotinemia report of case in adult, G D Head and R A Johnson	268

INDEX TO VOLUME 28

	PAGE
Cerebrospinal fluid, comparative studies in chemistry of blood and, G Egerer-Seham and C E Nixon	561
Chloroform, effect of cyanids and of organic oxidizing agents on liver injury caused by chloroform, N C Davis	20
Davidson, E C, and Dieuaide, F R Terminal cardiac arrhythmias, 3 cases	663
Davis, N C Effect of cyanids and of organic oxidizing agents on liver injury caused by chloroform	20
Dawson, P R, and Sullivan, M X Phenols in urine in pellagra	166
Dextrocardia, diagnosis of "eventration" of diaphragm, with report of case of aplasia of right lung and right half of diaphragm associated with congenital dextrocardia, H M Korns	192
Diabetes insipidus, experimental, P Bailey and F Bremer	773
insipidus, metabolic studies on, I M Rabinowitch	355
objects and method of diet adjustment in, R T Woodyatt	125
Diaphragm, diagnosis of "eventration" of, with report of case of aplasia of right lung and right half of diaphragm, associated with congenital dextrocardia, H M Korns	192
Diarrhea, etiology of an outbreak of infectious diarrhea, H Weiss	37
Dieuaide, F R, and Davidson, E C Terminal cardiac arrhythmias, 3 cases	663
Digitalis, experimental inquiry into cerebral and neuromuscular manifesta- tions of digitalis, D I Macht and W Bloom	678
Duke, W W Food allergy as cause of abdominal pain	151
Egerer-Seham, G, and Nixon, C E Comparative studies in chemistry of blood and cerebrospinal fluid	561
Electrocardiograms, study of S-T interval in 1,028 electrocardiograms, J A Buchanan	484
Ellis, A G Betelnut chewing and its effects, including cancer of mouth	252
Encephalitis, studies in oxygen therapy, an extreme type of shallow breath- ing occurring in lethargic encephalitis, A L Barach and M N Woodwell	421
Epitheliomata of thymic origin, D Symmers and B M Vance	239
Erythrocytes, studies on erythrocytes, with special reference to reticulum, polychromatophilia and mitochondria, J A Key	511
Fatigue, clinical studies of respiration, effect of general weakness and fatigue on vital capacity of lungs, F W Peabody and C C Sturgis	501
Fitz, R, and Beeler, C Glycemia, glycosuria and water excretion in obesity	804
Food allergy as cause of abdominal pain, W W Duke	151
Functional disease in soldiers, analysis of 90 cases, J M Swan	586
Gibson, R B, and Howard, C P Alkaptonuria with a study of its metabolism	632
Glycosuria, renal, P L Marsh	54
Gram, H C Causes of variations in sedimentation of corpuscles and formation of crista phlogistica ("size," "buffy coat") on blood, a preliminary communication	312
Guthrie, C C Simple and accurate metabolism spirometer, spirometer measurement of oxygen consumption by rebreathing method	841
Hay-fever, frequent causes and treatment of, I C Walker	71
local desensitization in hypersensitive individuals and its bearing on pre- vention of hay-fever, G M Mackenzie and L B Baldwin	722
Head, G D, and Johnson, R A Carotinemia, report of case in adult	268
Heart, bigeminal pulse in atrioventricular rhythm, P D White	213
further observations on experimental lesions of branches of auriculo- ventricular bundle of dog, F M Smith	453
perforation of interventricular septum of heart, with report of case, J B Youmans	495
right ventricle in pulmonary tuberculosis, E P Boas and H Mann	62

INDEX TO VOLUME 28

	PAGE
Hemal nodes, primary nemangiolymproma of hemal nodes an unusual variety of malignant tumor, D Symmers	467
Hemangiolymproma, primary hemangiolymproma of hemal nodes an unusual variety of malignant tumor, D Symmers	467
Howard, C P, and Gibson, R B Alkaptonuria with a study of its metabolism	632
Infection focal, and elective localization in etiology of myositis, E C Rosenow and W Ashby	274
Johnson, R A and Head, G D Carotinemia, report of case in adult	268
Kahn, M, and Barsky, J Acute yellow atrophy of liver	142
Kast L and Killian, J A Study of significant chemical changes in blood coincident with malignant tumors	813
Key, J A Studies on erythrocytes, with special reference to reticulum, polychromatophilia and mitochondria	511
Kidney function, study of urea concentration test for kidney function, I M Rabmowitch	827
function, synthesis and elimination of hippuric acid in nephritis a new renal function test, F B Kingsbury and W W Swanson	220
irritation in man from high protein diet, T L Squier and L H Newburgh	1
new method of interpretation of renal function test meal, H Sharlit and W G Lyle	649
Killian, J A, and Kast, L Study of significant chemical changes in blood coincident with malignant tumors	813
Kingsbury, F B, and Swanson, W W Synthesis and elimination of hippuric acid in nephritis a new renal function test	220
Korns, H M Diagnosis of "eventration" of diaphragm, with report of case of aplasia of right lung and right half of diaphragm, associated with congenital dextrocardia	192
Leter, L Relation of urea to uremia	331
Lewis, N D C Study of tumors from primitive notochord	434
Liver, acute yellow atrophy of, M Kahn and J Barsky	142
disease, basal metabolism and specific dynamic action of protein in, J C Aub and J H Means	173
injury, effect of cyanids and of organic oxidizing agents on, caused by chloroform, N C Davis	20
Lung cancer, primary, T S Moise	733
diagnosis of "eventration" of, with report of case of aplasia of right lung and right half of diaphragm, associated with congenital dextrocardia, H M Korns	192
Lyle, W G, and Sharlit, H New method of interpretation of renal function test meal	649
McCann, W S Effect of ingestion of foodstuffs on respiratory exchange in pulmonary tuberculosis	847
Macht, D I, and Bloom, W Experimental inquiry into cerebral and neuromuscular manifestations of digitalis	678
Mackenzie, G M, and Baldwin, L B Local desensitization in hypersensitive individuals and its bearing on prevention of hay-fever	722
McKinlay, C A Effect of extract of posterior lobe of pituitary on basal metabolism in normal individuals and in those with endocrine disturbances	703
Mann H, and Boas, E P Right ventricle in pulmonary tuberculosis	62
Marsh, P L Renal glycosuria	54
Means, J H, and Aub J C Basal metabolism and specific dynamic action of protein in liver disease	173
Metabolism, basal, and specific dynamic action of protein in liver disease, J C Aub and J H Means	173
basal, effect of extract of posterior lobe of pituitary on basal metabolism in normal individuals and in those with endocrine disturbances, C A McKinlay	703

Metabolism—Continued

effect of ingestion of foodstuffs on respiratory exchange in pulmonary tuberculosis, W S McCann and G F Soderstrom	847
simple and accurate metabolism spirometer, spirometer measurement of oxygen consumption by rebreathing method, C C Guthrie	841
studies on case of diabetes insipidus, I M Rabinowitch	355
Minot, G R, and Smith, L W Blood in tetrachlorethane poisoning	687
Moise T S Primary carcinoma of lungs	733
Mouth, betelnut chewing and its effects, including cancer of mouth, A G Ellis	252
Musser, J H, Jr Influence of inorganic iron on regeneration of blood after hemorrhagic anemia	638
Myositis, focal infection and elective localization in etiology of myositis, E C Rosenow and W Ashby	274
Nephritis, interstitial, variation of phenolsulphonephthalein excretion with the urine volume in, R R Snowden	603
synthesis and elimination of hippuric acid in nephritis a new renal function test, F B Kingsbury and W W Swanson	220
total nonprotein nitrogen constituents of blood in chronic nephritis with hypertension, J L Williams	426
uncinariar nephritis, F Rojas and J Tanca Morengo	550
Newburgh, L H and Squier, T L Renal irritation in man from high protein diet	1
Nitrites, action of, on coronary circulation, F M Smith	836
Nixon, C E, and Egerer-Seham, G Comparative studies in chemistry of blood and cerebrospinal fluid	561
Notochord, contribution to study of tumors from the primitive notochord, N D C Lewis	434
Obesity, observations on glycemia, glycuressis and water excretion in obesity, C Beeler and R Fitz	804
Oxygen therapy, studies in oxygen therapy with determinations of blood gases, in cardiac insufficiency and related conditions, A L Barach and M N Woodwell	367
therapy, studies in oxygen therapy, in pneumonia and its complications, A L Barach and M N Woodwell	394
therapy, studies in oxygen therapy, an extreme type of shallow breathing occurring in lethargic encephalitis, A L Barach and M N Woodwell	421
Peabody, F W, and Sturgis, C C Clinical studies of respiration VII Effect of general weakness and fatigue on vital capacity of lungs	501
Pellagra, phenols in urine in pellagra, M X Sullivan and P R Dawson	166
some urinary changes in normal individuals on pellagra producing experimental diet, M X Sullivan	119
Pellini, E J, and Wallace, G B Capillary poisons and acidosis	711
Phenols in urine in pellagra, M X Sullivan and P R Dawson	166
Pituitary extract, effect of extract of posterior lobe of pituitary on basal metabolism in normal individuals and in those with endocrine disturbances, C A McKinlay	703
Pneumonia, studies in oxygen therapy, in pneumonia and its complications, A L Barach and M N Woodwell	394
Protein, renal irritation in man from high protein diet, T L Squier and L H Newburgh	1
therapy, intravenous use of foreign protein in treatment of chronic cases of arthritis, with special reference to use of secondary proteose, R G Snyder and M A Ramirez	50
Pulse, bigeminal pulse in atrioventricular rhythm, P D White	213
Rabinowitch, I M Metabolic studies on case of diabetes insipidus	355
Study of urea concentration test for kidney function	827
Ramirez, M A, and Snyder, R G Intravenous use of foreign protein in treatment of chronic cases of arthritis, with special reference to use of secondary proteose	50

Respiration, clinical studies of respiration, effect of general weakness and fatigue on vital capacity of lungs, F W Peabody and C C Sturgis	501
Rojas, F and Tanca Morengo, J Uncinarial nephritis	550
Rosenow E C, and Ashby W Focal infection and elective localization in etiology of myositis	274
Sharlit, H, and Lyle W G New method of interpretation of renal function test meal	649
Smith, F M Action of nitrites on coronary circulation	836
Experimental lesions of branches of auriculoventricular bundle of dog	453
Smith L W and Minot, G R Blood in tetrachlorethane poisoning	687
Snowden, R R Variation of phenolsulphonaphthalein excretion with urine volume in chronic interstitial nephritis	603
Snyder R G, and Ramirez M A Intravenous use of foreign protein in treatment of chronic cases of arthritis, with special reference to use of secondary proteose	50
Soderstrom, G F, and McCann, W S Effect of ingestion of foodstuffs on respiratory exchange in pulmonary tuberculosis	847
Spirometer, simple and accurate metabolism spirometer, spirometer measurement of oxygen consumption by rebreathing method, C C Guthrie	841
Squier, T L, and Newburgh, L H Renal irritation in man from high protein diet	1
Stomach, relation of gastric content to secretory and motor functions of stomach, H Wheelon	613
Sturgis C C, and Peabody, F W Clinical studies of respiration VII Effect of general weakness and fatigue on vital capacity of lungs	501
Sullivan, M X Urinary changes in normal individuals on the pellagra producing experimental diet	119
and Dawson, P R Phenols in urine in pellagra	166
Swan, J M Analysis of 90 cases of functional disease in soldiers	586
Swanson, W W, and Kingsbury, F B Synthesis and elimination of hippuric acid in nephritis a new renal function test	220
Symmers, D Primary hemangiolymphoma of hernal nodes an unusual variety of malignant tumor	467
and Vance, B M Epitheliomata of thymic origin	239
Tanca Morengo, J, and Rojas, F Uncinarial nephritis	550
Test meal, new method of interpretation of renal function test meal, H Sharlit and W G Lyle	649
Tetrachlorethane poisoning, blood in, G R Minot and L W Smith	687
Thymus, epitheliomata of thymic origin, D Symmers and B M Vance	239
Tuberculosis, pulmonary, effect of ingestion of foodstuffs on respiratory exchange in pulmonary tuberculosis, W S McCann and G F Soderstrom	847
pulmonary, right ventricle in, E P Boas and H Mann	62
Tumor, primary hemangiolymphoma of hernal nodes an unusual variety of malignant tumor, D Symmers	467
contribution to study of tumors from the primitive notochord, N D C Lewis	434
study of significant chemical changes in blood coincident with malignant tumors, J A Killian and L Kast	813
Urea, relation of urea to uremia, L Leiter	331
Uremia, relation of urea to uremia, L Leiter	331
Urine, phenols in urine in pellagra, M X Sullivan and P R Dawson	166
some urinary changes in normal individuals on pellagra producing experimental diet, M X Sullivan	119
Urobilin, chemistry and clinical significance of, L Bauman	475
Vance, B M, and Symmers, D Epitheliomata of thymic origin	239
Vital capacity of lungs, effect of general weakness and fatigue on, F W Peabody and C C Sturgis	501

INDEX TO VOLUME 28

	PAGE
Walker, I C Frequent causes and treatment of seasonal hay-fever	71
Wallace G B, and Pellini, E J Capillary poisons and acidosis	711
Weiss, H On etiology of an outbreak of infectious diarrhea	37
Wheelon, H Relation of gastric content to secretory and motor functions of stomach	613
White, P D Bigeminal pulse in atrioventricular rhythm	213
Williams, J L Total nonprotein nitrogen constituents of blood in chronic nephritis with hypertension	426
Woodwell, M N, and Barach, A L Studies in oxygen therapy with determinations of blood gases I In cardiac insufficiency and related conditions	367
Studies in oxygen therapy II In pneumonia and its complications	394
Studies in oxygen therapy III In an extreme type of shallow breathing occurring in lethargic encephalitis	421
Woodyatt, R T Objects and method of diet adjustment in diabetes	125
Youmans, J B Perforation of interventricular septum of heart	495

Fifty cents each will be paid for the following issues of the Archives of Internal Medicine January, March, June, August, 1918 January and July, 1916, November, 1915, January, 1911, July, 1909 AMERICAN MEDICAL ASSOCIATION, 535 North Dearborn Street, Chicago, Ill
